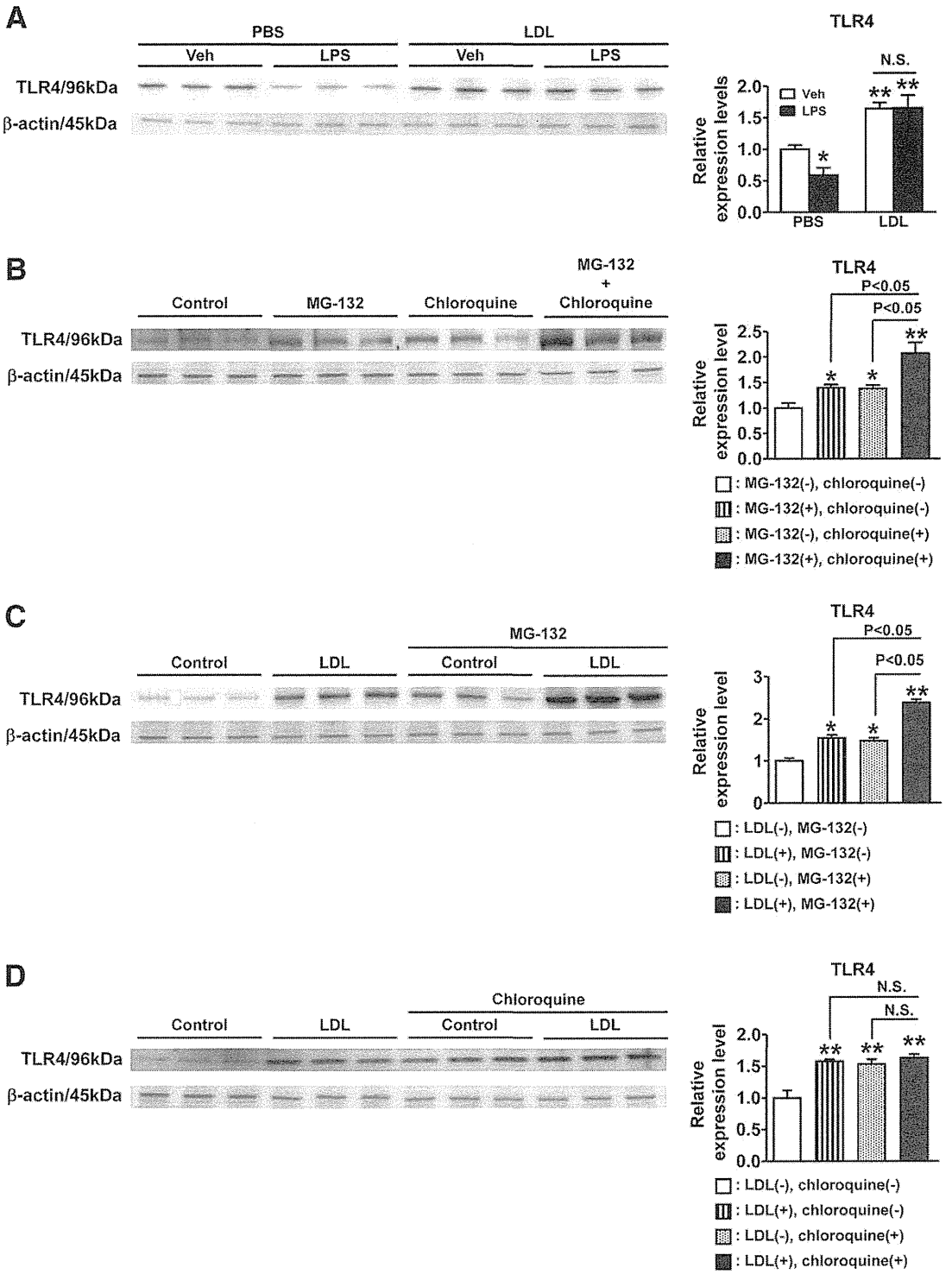

Fig. 4. FC accumulation in HSCs enhances TLR4 protein expression by suppressing the endosomal-lysosomal degradation pathway of TLR4. (A) Expression and quantification of TLR4 protein expression in vehicle-treated or LDL-treated HSCs, 60 minutes after addition of LPS (100 ng/mL), compared with cells not treated with LPS. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the control culture, without LPS treatment. (B) Expression and quantification of TLR4 protein expression in HSCs treated with MG-132 and/or chloroquine. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the control culture. (C) Expression and quantification of TLR4 protein expression in HSCs treated with LDL in the presence/absence of MG-132. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the control culture. (D) Expression and quantification of TLR4 protein expression in HSCs treated with LDL in the presence/absence of chloroquine. $^{**}P < 0.01$, compared with the control culture. All data are expressed as means (SEM).

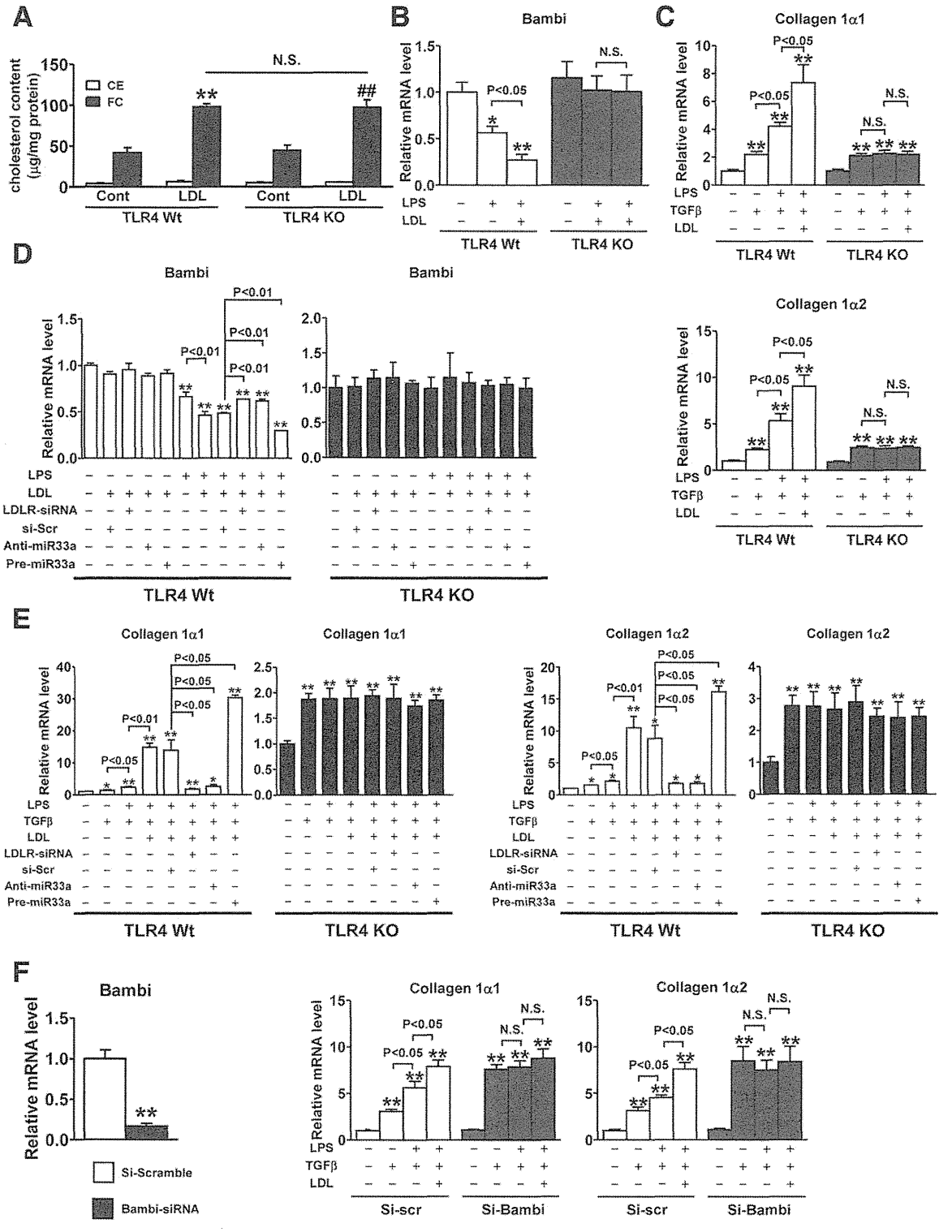

Fig. 5. FC accumulation in HSCs sensitizes HSCs to TGFβ-induced activation through enhancement of TLR4-mediated down-regulation of Bambi. (A) Quantification of cellular FC and CE in wild-type or TLR4-deficient HSCs, treated or untreated with LDL. $^{**}P < 0.01$ and $^{\#\#}P < 0.01$, compared with the corresponding control culture. (B) Quantification of Bambi mRNA in wild-type or TLR4-deficient HSCs, treated with LPS and/or LDL. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the corresponding control culture. (C) Quantification of collagen 1α1 and collagen 1α2 mRNA in wild-type or TLR4-deficient HSCs, treated with LPS, TGFβ, and/or LDL. $^{**}P < 0.01$, compared with the corresponding control culture. (D) Quantification of Bambi mRNA in wild-type or TLR4-deficient HSCs, treated with LDLR-siRNA, control-siRNA, anti-miR33a, or pre-miR33a in the presence of LPS and/or LDL. $^{**}P < 0.01$, compared with the corresponding control culture. (E) Quantification of collagen 1α1 and collagen 1α2 mRNA in wild-type or TLR4-deficient HSCs, treated with LDLR-siRNA, control-siRNA, anti-miR33a, or pre-miR33a in the presence of LPS, TGFβ, and/or LDL. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the corresponding control culture. (F) Quantification of Bambi mRNA in HSCs treated with Bambi-siRNA or control-siRNA (left panel). Quantification of collagen 1α1 and collagen 1α2 mRNA in wild-type HSCs, treated with Bambi-siRNA or control-siRNA in the presence of LPS, TGFβ, and/or LDL (right panel). $^{**}P < 0.01$, compared with the corresponding control culture. All data are expressed as means (SEM).

(Fig. 5D). This decrease was significantly enhanced in cells treated with LDL, whereas treatment with LDLR-siRNA reversed the LDL-induced decrease in Bambi mRNA expression (Fig. 5D). Similarly, treatment with anti-miR33a reversed the LDL-induced decrease in Bambi mRNA expression. On the other hand, treatment with pre-miR33a enhanced the LDL-induced decrease in Bambi mRNA expression (Fig. 5D). These results were in accordance with the results of FC accumulation and TLR4 protein expression in HSCs, and a deficiency in TLR4 signaling reversed all these changes (Fig. 5D).

Treatment with LDLR-siRNA reversed the LDL-induced increase in the mRNA expressions of collagen 1α1 and 1α2 in wild-type HSCs treated with LPS and TGFβ (Fig. 5E). In accordance with the results of FC accumulation and Bambi mRNA expression in HSCs, treatment with anti-miR33a reversed the LDL-induced increase in collagen 1α1 and 1α2 mRNA expression and treatment with pre-miR33a enhanced it (Fig. 5E). As is the case in Bambi mRNA expression, a deficiency in TLR4 signaling canceled all these LDL-induced changes in collagen 1α1 and 1α2 mRNA expression (Fig. 5E). In addition, treatment with Bambi-siRNA reversed the LDL-induced increase in the mRNA expression of collagen 1α1 and 1α2 in HSCs treated with LPS and TGFβ (Fig. 5F). Furthermore, in the same way as in the *in vitro* study, treatment with antagomirs against miR33a significantly alleviated the activation of HSCs in the mouse model of liver fibrosis induced by carbon tetrachloride (CCl_4). This occurred through the suppression of FC accumulation and the subsequent inhibition of TLR4-mediated down-regulation of Bambi in HSCs (Supporting Fig. 8).

Increased Intake of Cholesterol Does Not Impact Liver Fibrosis in NASH in TLR4-Deficient Mice. We used TLR4-deficient mice to assess whether the exacerbation of liver fibrosis in NASH by increased cholesterol intake was dependent on TLR4 signal transduction. Significant differences were not observed in the extent of liver fibrosis or in the hepatic mRNA levels of collagen 1α1, collagen 1α2, and αSMA, between MCD diet-fed and MCD+HC diet-fed TLR4-deficient mice (Fig. 6A-C). Similarly, the increased cholesterol intake did not enhance liver fibrosis in the HF diet-induced NASH in TLR4-deficient mice (Fig. 6D-F).

SREBP2-Mediated Feedback Regulation of Cholesterol Homeostasis Is Disrupted in HSCs and HSC Activation Further Enhances the Disruption. Nuclear accumulation of hepatic SREBP2 decreased in the two mouse models of NASH and further declined following supplementation with cholesterol (Supporting Fig. 9A). Cholesterol supplementation significantly decreased the hepatic mRNA levels of LDLR and HMGCR, which are downstream molecules of SREBP2, in both the animal models (Supporting Fig. 9B,C).

We next detailed the SREBP2-mediated feedback system of cholesterol homeostasis in hepatocytes and HSCs *in vitro*. The nuclear form of SREBP2 in hepatocytes was dramatically decreased by treatments with LDL (Fig. 7A) and 25-hydroxycholesterol, which promotes Scap-Insig complex formation.[11] These treatments also significantly decreased the nuclear form of SREBP2 in quiescent HSCs but did not affect that in activated HSCs (Fig. 7A). Quantitative analysis showed that the decrease was significantly enhanced in hepatocytes, compared with HSCs, and quiescent HSCs, compared with activated HSCs (Fig. 7A).

MβCD reportedly delivers cholesterol to cells without passing through lysosomes.[12] Treatment with a cholesterol-MβCD complex also dramatically decreased the nuclear form of SREBP2 in hepatocytes (Fig. 7A). This treatment significantly decreased the nuclear form of SREBP2 in quiescent HSCs but did not affect that in activated HSCs (Fig. 7A). Quantitative analysis showed that the decrease was significantly enhanced in hepatocytes, compared with HSCs, and in quiescent HSCs, compared with activated HSCs (Fig. 7A). Scap expression levels were much higher in quiescent and activated HSCs than in hepatocytes (Fig. 7B). However, the Insig-1 expression level in hepatocytes was comparable to that in quiescent HSCs; we did not detect any expression of Insig-1 in activated HSCs (Fig. 7B). Hepatocytes expressed Insig-2 protein, whereas we could not observe any expression of Insig-2 in HSCs (Fig. 7B).

A Scap trypsin cleavage assay[13] was subsequently performed to examine whether or not cholesterol-induced Scap conformational changes occurred in these cells. Scap, without cholesterol-induced conformational changes, yields a protected band of 27 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), whereas Scap, with the conformational change, yields a protected band of 26 kDa. Our data showed that the cholesterol-induced Scap conformational change in activated HSCs occurred to the same degree as that in quiescent HSCs or hepatocytes (Supporting Fig. 10A,B).

LDL treatment decreased the nuclear level of SREBP2 in quiescent HSCs. Treatment with Scap-siRNA or Insig-2-overexpression vector enhanced the effect, whereas treatment with Insig-1-siRNA

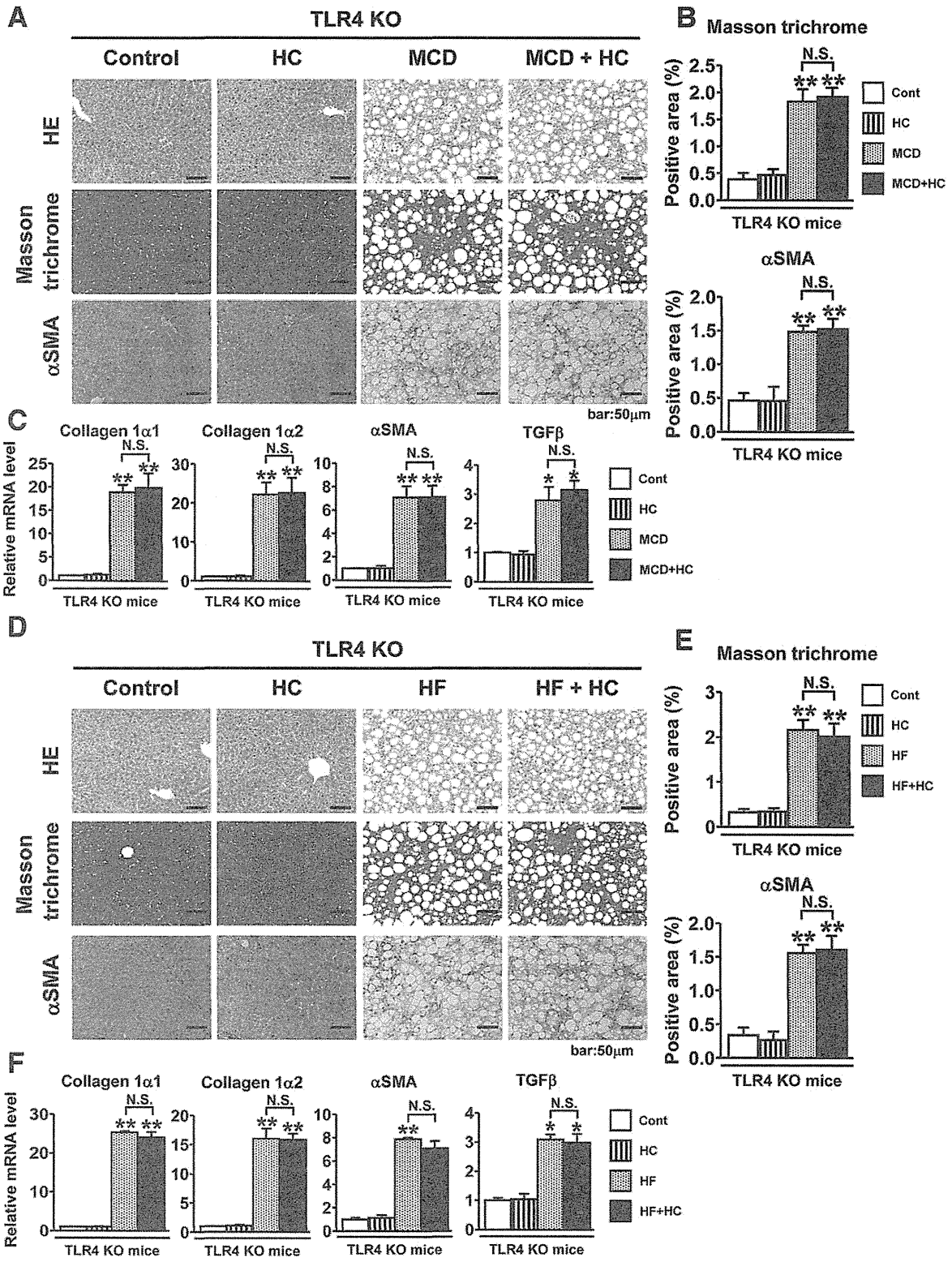

Fig. 6. Increased cholesterol intake does not impact liver fibrosis in NASH in TLR4-deficient mice. TLR4-deficient mice (7-8 weeks old; n = 4-7/group) were fed (A-C) the control, HC, MCD, or MCD+HC diet for 8 weeks or (D-F) the control, HC, HF, or HF+HC diet for 20 weeks. (A,D) Hematoxylin and eosin-stained, Masson's trichrome-stained, and αSMA-immunostained sections of representative liver samples. (B,E) Quantification of Masson's trichrome staining (upper panel) and αSMA immunostaining (lower panel). (C,F) Quantification of hepatic collagen 1α1, collagen 1α2, αSMA, and TGFβ mRNA. $^{**}P < 0.01$, compared with the control diet group. All data are expressed as means (SEM).

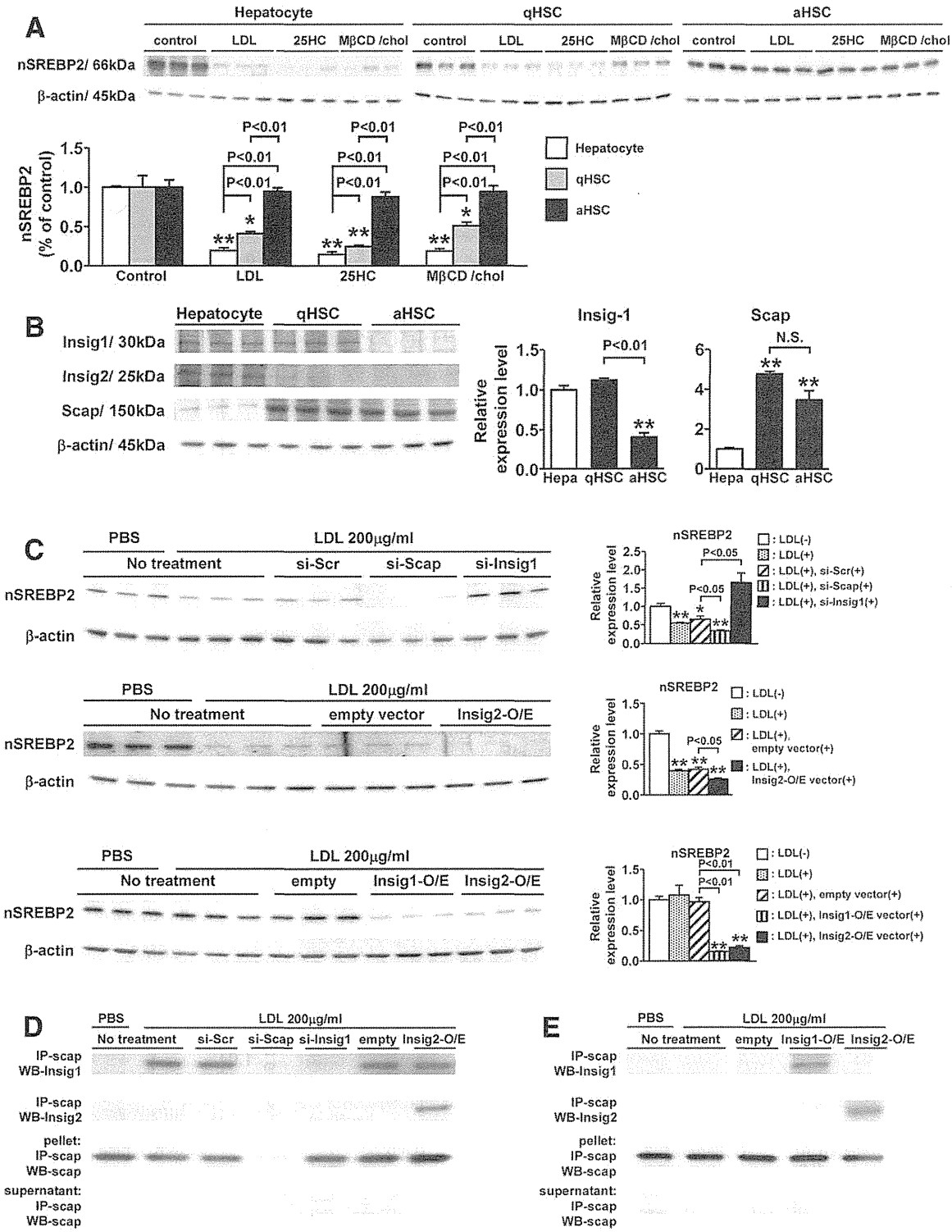

Fig. 7. The sterol regulatory systems in HSCs are disrupted and dependent on the relative amounts of Scap and Insigs. (A) Expression and quantification of the nuclear form of SREBP2 protein in hepatocytes, quiescent HSCs (qHSCs; cultured for 1 day after isolation), and activated HSCs (aHSCs; cultured for 7 days after isolation) after treatment with LDL, 25-hydroxycholesterol (25-HC), or MβCD/cholesterol complex. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the corresponding control culture. (B) Expression and quantification of Insig-1, Insig-2, and Scap protein in hepatocytes, qHSCs, and aHSCs. $^{**}P < 0.01$, compared with the levels in hepatocytes. (C) Expression and quantification of the nuclear form of SREBP2 protein (upper panel) in qHSCs treated with Scap-siRNA, Insig-1-siRNA, or control-siRNA in the presence/absence of LDL, (middle panel) in qHSCs treated with Insig-2-O/E vector or control vector in the presence/absence of LDL, (lower panel) in aHSCs treated with Insig-1-O/E vector, Insig-2-O/E vector, or control vector in the presence/absence of LDL. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the control culture. (D) Immunoprecipitation analysis of Scap-Insig-1 and Scap-Insig-2 complexes in qHSCs treated with control-siRNA, Scap-siRNA, Insig-1-siRNA, control vector, or Insig-2-O/E vector in the presence of LDL. (E) Immunoprecipitation analysis of Scap-Insig-1 and Scap-Insig-2 complexes in aHSCs treated with control vector, Insig-1-O/E vector, or Insig-2-O/E vector in the presence of LDL. All data are expressed as means (SEM).

counteracted the effect (Fig. 7C, upper and middle). However, LDL treatment did not affect the nuclear level of SREBP2 in activated HSCs; overexpression of Insig-1 or Insig-2 in HSCs significantly decreased the nuclear level of SREBP2 after the addition of LDL (Fig. 7C, lower).

LDL treatment increased the level of the Scap-Insig-1 complex in quiescent HSCs, whereas cotreatment with Scap-siRNA or Insig-1-siRNA reversed this change (Fig. 7D). We could not detect any Scap-Insig-2 complex in quiescent HSCs after the addition of LDL. Overexpression of Insig-2 increased the level of the Scap-Insig-2 complex in LDL-treated quiescent HSCs (Fig. 7D). On the other hand, neither the Scap-Insig-1 nor the Scap-Insig-2 complex could be detected in activated HSCs treated with LDL or not (Fig. 7E). Overexpression of Insig-1 increased the level of the Scap-Insig-1 complex in activated HSCs treated with LDL, and similarly, overexpression of Insig-2 increased the level of the Scap-Insig-2 complex after treatment with LDL (Fig. 7E).

In addition, the feedback regulation system of cholesterol homeostasis impacted the sensitization of HSCs to TGFβ-induced activation, in a manner similar to the FC accumulation system mediated by LDLR or miR33a (Supporting Fig. 11).

HSC Activation in NASH Down-Regulates Insig-1 Expression Through the Suppression of PPARγ Signal Transduction. The Insig-1 expression level was significantly lower in HSCs from the MCD and HF diet-fed groups than in those from the corresponding control diet-fed groups (Fig. 8A,B; Supporting Fig. 12A,B). These decreases were significantly enhanced by the increased intake of cholesterol (Fig. 8A,B; Supporting Fig. 12A,B). We could not detect any difference in the Scap expression level in HSCs among the groups (Fig. 8A,B; Supporting Fig. 12A,B).

Furthermore, Insig-1 protein was abundant in quiescent HSCs but its level declined at days 3 and 5, and day 7 HSCs (Supporting Fig. 12C). We could not detect any significant difference in the Scap expression level among the groups (Supporting Fig. 12C). Similar results were obtained in terms of the mRNA expression levels of Insig-1 and Scap (Supporting Fig. 12C). Treatment with the PPARγ antagonist significantly decreased the Insig-1 expression level in quiescent HSCs in a dose-dependent manner (Fig. 8C).

Discussion

This study showed that increased cholesterol intake accelerated liver fibrosis in the two mouse models of NASH without affecting the degree of hepatocellular injury or Kupffer cell activation. The exacerbation of liver fibrosis mainly involved FC accumulation in HSCs, which increased TLR4 protein levels through suppression of the endosomal-lysosomal degradation pathway of TLR4, down-regulated the expression of the TGFβ pseudoreceptor Bambi, and thereby sensitized the cells to TGFβ-induced activation. This study also showed that FC loading of HSCs is not sufficient to induce activation but serves to enhance activation initiated by TGFβ. These results are compatible with our previous finding[3] that showed that FC accumulation in HSCs increased membrane TLR4 levels; suppressed the HSC expression of Bambi, the TLR4 target gene[14]; and subsequently exaggerated liver fibrosis in mouse models of liver fibrosis.

This study also helped to elucidate the main mechanisms by which HSCs are sensitive to FC accumulation. The SREBP2-mediated feedback system, which plays a major role in maintaining cellular cholesterol homeostasis,[5,6] was disrupted in HSCs; this disruption could be attributed to high expression of Scap and no expression of Insig-2 in these cells. This could explain why the HC diet significantly reduced SREBP2 signaling in hepatocytes but not in HSCs, and resulted in enhanced FC accumulation in HSCs.

Furthermore, HSC activation sensitized these cells to FC accumulation. Repression of PPARγ signaling underlies HSC transdifferentiation.[15] In the present study, the level of PPARγ decreased along with the activation of HSCs. The suppression of PPARγ signaling in activated HSCs decreased the cellular expression of Insig-1, which resulted in enhancing the disruption of the SREBP2-mediated cholesterol-feedback system. This could partly explain why SREBP2 signaling in HSCs was enhanced, along with their activation, although FC accumulation continued to increase.

In addition, the decreased PPARγ signaling in activated HSCs also enhanced SREBP2 expression and signaling, resulting in enhanced expression of the LDLR, the SREBP2 target gene, in HSCs. As *SREBF2* is a bifunctional locus encoding SREBP2 and miR-33a,[10] suppression of PPARγ signaling also increased the level of miR-33a in HSCs, in turn suppressing the levels of NPC1 and ABCA1 (data not shown), which are negatively regulated by miR-33a.[10] These results showed that HSC activation enhanced FC accumulation, in part because of the increased LDLR level and the decreased NPC1 and ABCA1 levels.

The present results suggest that these characteristic mechanisms in HSCs could sensitize the cells to enhanced FC accumulation after increased intake of

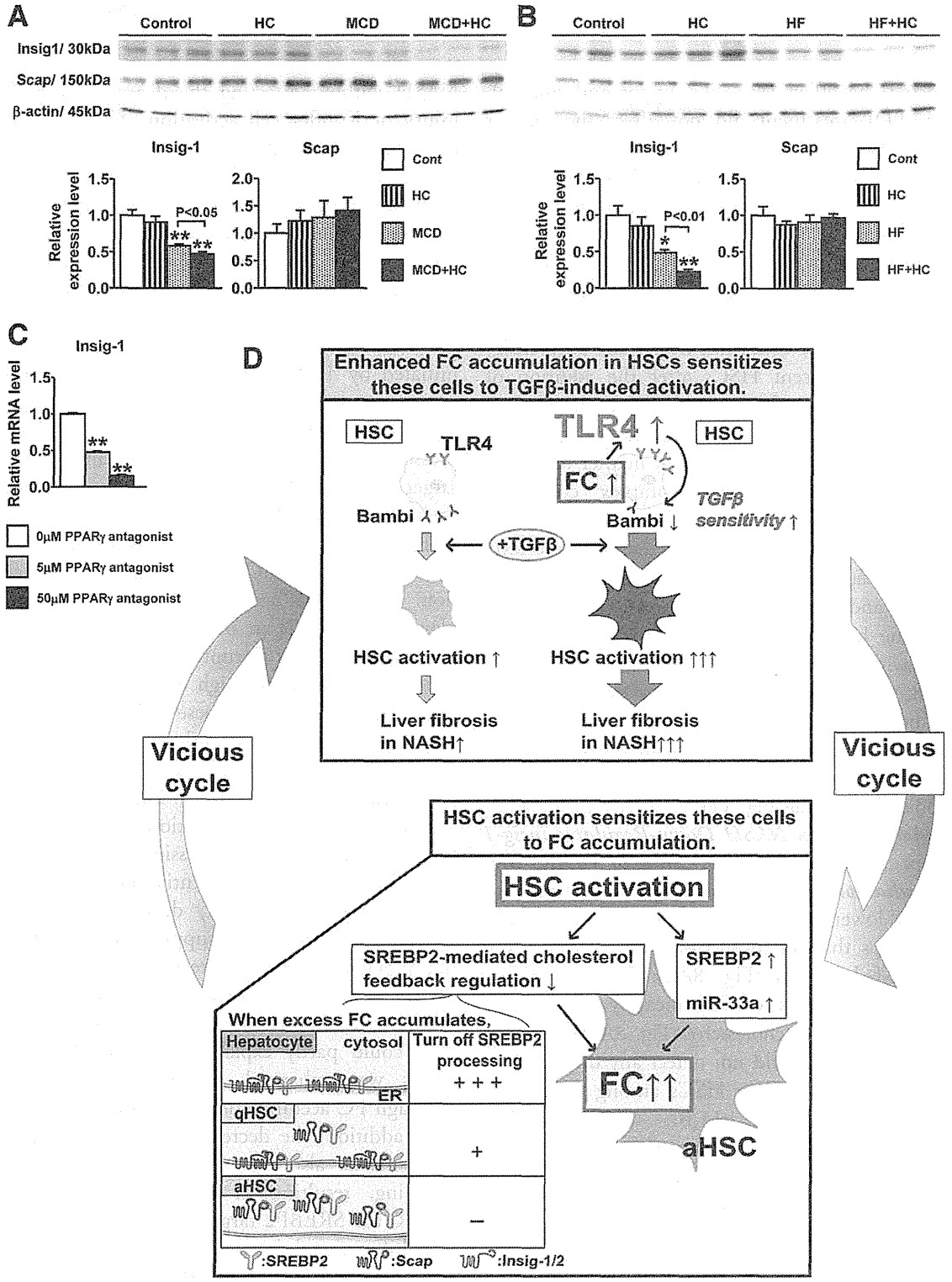

Fig. 8. Down-regulation of Insig-1 expression by HSC activation through the suppression of PPARγ signal transduction. C57BL/6 mice (9 weeks old, male; n = 6-9/group) were fed (A) the control, HC, MCD, or MCD+HC diet for 12 weeks or (B) the control, HC, HF, or HF+HC diet for 24 weeks. (A,B) Expression and quantification of Insig-1 and Scap protein in HSCs isolated from the mice in each group. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with the control diet group. (C) Quantification of Insig-1 mRNA in quiescent HSCs treated with the PPARγ antagonist. $^{**}P < 0.01$, compared with the control culture. All data are expressed as means (SEM). (D) Schematic of the characteristic mechanisms of FC accumulation in HSCs during the development of liver fibrosis in NASH. FC loading of HSCs is not sufficient to induce activation but serves to enhance activation initiated by TGFβ. Enhanced FC accumulation in HSCs plays an important role in the progression of liver fibrosis in NASH by promoting TLR4 signal transduction through suppression of the endosomal-lysosomal degradation pathway of TLR4, down-regulating the Bambi expression level, and subsequently sensitizing HSCs to TGFβ-induced activation. HSCs are sensitive to FC accumulation because of the high intracellular Scap-to-Insig expression ratio, and furthermore, HSC activation dysregulates their cholesterol metabolism, resulting in further FC accumulation and exaggerating liver fibrosis in a vicious cycle.

cholesterol and/or activation of HSCs. They also suggest that such accumulation could play an important role as a mediator of the vicious cycle of HSC activation in NASH (Fig. 8D).

There are two major pathways for cell surface receptor degradation after ubiquitination: a ubiquitin-proteasome pathway and a lysosomal degradation pathway.[16] Our present results showed that FC accumulation in HSCs inhibited the degradation of TLR4, mainly by down-regulating a lysosomal degradation pathway, which resulted in increased levels of TLR4 protein. These results are compatible with our previous report[3] showing that FC accumulation in HSCs could be involved in endosomal-lysosomal dysfunction.

The MCD diet-induced mouse model is commonly used as a model of NASH, and the resulting characteristic pathology of steatosis, mixed cell inflammatory infiltrate, hepatocellular necrosis, and pericellular fibrosis mimics that found in humans with NASH.[17,18] Nevertheless, the mice do not develop the accompanying metabolic syndrome that is often associated with human NASH. Therefore, we also used an HF diet-induced model of NASH to examine the precise role of cholesterol in the pathophysiology of NASH. As the results were similar in both mouse models of NASH, our findings may indicate a role for cholesterol in the pathophysiology of NASH.

Mari et al.[19] reported that mitochondrial FC loading accounted for hepatocellular sensitivity to TNFα. Furthermore, they showed that the mitochondrial FC content in mouse hepatocytes increased transiently only during the first 6 days of HC feeding, and thereafter returned to its prior level.[19] Our results also showed that chronic HC feeding did not significantly increase mitochondrial FC accumulation in hepatocytes. This could be one reason why an increased intake of cholesterol did not impact the hepatocellular damage in our two mouse models of NASH.

A recent report showed that accumulation of cholesterol in the lysosomes of Kupffer cells increased hepatic inflammation in the mouse model of NAFLD.[20] 27-Hydroxycholesterol is enzymatically generated from mitochondrial cholesterol by the mitochondrial P450 enzyme, Cyp27a1.[21] Further, it mobilizes cholesterol from the lysosomes to the cytoplasm, resulting in a reduction in the accumulation of lysosomal cholesterol in Kupffer cells.[20] In both mouse models of NASH, an increased intake of cholesterol did not affect the lysosomal cholesterol levels in Kupffer cells, nor did it impact the mitochondrial cholesterol levels or Cyp27a1 expression levels in Kupffer cells. These could be some reasons why increased cholesterol intake did not accelerate Kupffer cell activation in our mouse models of NASH.

In conclusion, FC accumulation in HSCs was enhanced mainly by two mechanisms: enhancement of both SREBP2 and miR-33a signaling through the suppression of PPARγ signaling along with HSC activation and disruption of the SREBP2-mediated cholesterol-feedback system in HSCs, which was characterized by a high Scap-to-Insig ratio and exaggerated by the down-regulation of Insig-1 through the suppression of PPARγ signaling along with HSC activation. Enhanced FC accumulation in HSCs plays an important role in the progression of liver fibrosis in NASH by promoting TLR4 signal transduction through suppression of the endosomal-lysosomal degradation pathway of TLR4, and subsequently sensitizing HSCs to TGFβ-induced activation. HSC activation dysregulates their cholesterol metabolism, resulting in further FC accumulation and exaggerating liver fibrosis in a vicious cycle (Fig. 8D). We believe that the characteristic mechanisms of FC accumulation in HSCs should be further studied as potential targets to treat liver fibrosis in liver diseases including NASH.

Acknowledgment: The authors thank Mina Kitazume and Miho Takabe (Keio University) for helpful advice and technical assistance, and Drs. Ikuo Inoue and Makoto Seo (Saitama Medical School) for helpful discussion and critical comments.

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Gastroenterology 2014;146:96–109

CLINICAL AT

Subcutaneous Golimumab Maintains Clinical Response in Patients With Moderate-to-Severe Ulcerative Colitis

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This article has an accompanying continuing medical education activity on page e14. Learning Objective: Upon completion of this CME activity, successful learners will be able to formulate a treatment plan which employs anti-tumor necrosis factor therapy with golimumab in patients with moderate to severe ulcerative colitis.

Podcast interview: www.gastro.org/gastropodcast. Also available on iTunes. See Covering the Cover synopsis on page 1; see editorial on page 13; see related article, Sandborn WJ et al, on page 85.

BACKGROUND & AIMS: Subcutaneous golimumab, a fully human monoclonal antibody to tumor necrosis factor-α (TNFα), was evaluated as maintenance therapy in TNFα antagonist-naive adults with moderate-to-severe active ulcerative colitis, despite conventional therapy, who responded to golimumab induction therapy. **METHODS:** We performed a phase 3, double-blind trial of patients who completed golimumab induction trials (Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment, eg, PURSUIT). Patients who responded to induction therapy with golimumab (n = 464) were assigned randomly to groups given placebo or injections of 50 or 100 mg golimumab every 4 weeks through week 52. Patients who responded to placebo in the induction study continued to receive placebo. Nonresponders in the induction study received 100 mg golimumab. The primary end point was clinical response maintained through week 54; secondary end points included clinical remission and mucosal healing at both weeks 30 and 54. **RESULTS:** 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 ($P = .010$ and $P < .001$, 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%; $P = .004$ and $P = .002$, respectively) or 50 mg golimumab (23.2% and 41.7%, respectively). Percentages of serious adverse events were 7.7%, 8.4%, and 14.3% among patients given placebo, 50 mg, or 100 mg golimumab, respectively; percentages of serious infections were 1.9%, 3.2%, and 3.2%, respectively. Among all patients given golimumab in the study, 3 died (from sepsis, tuberculosis, and cardiac failure, all in patients who received 100 mg golimumab) and 4 developed active tuberculosis. **CONCLUSIONS:** Golimumab (50 mg or 100 mg) maintained clinical response through week 54 in patients who responded to induction therapy with golimumab and had moderate-to-severe active ulcerative colitis; patients who received 100 mg golimumab had clinical remission and mucosal healing at weeks 30 and 54. Safety was consistent with that reported for other TNFα antagonists and golimumab in other approved indications. ClinicalTrials.gov number: NCT00488631.

Keywords: Inflammatory Bowel Disease; Fully Human Monoclonal Antibody; Randomized Withdrawal Trial.

Ulcerative colitis (UC) is a chronic disease characterized by recurrent or continuous uncontrolled inflammation of the colon.[1] The extent and severity of disease can range from mild proctitis to fulminant extensive

Abbreviations used in this paper: **IV, intravenous; NNT, number needed to treat; PURSUIT, Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment; PURSUIT-M, Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment Maintenance; SC, subcutaneous; TNFα, tumor necrosis factor-α; UC, ulcerative colitis.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2013.06.010

disease. The most common course of UC is recurrent attacks of bloody diarrhea interspersed with periods of remission.[2] Symptoms can include frequent evacuations of blood and mucus, fecal urgency caused by reduced rectal compliance, incontinence, abdominal pain, weight loss, and general malaise.[1] Colectomy remains a significant outcome for UC patients with rates varying by disease severity and duration.[3–5]

The tumor necrosis factor-α (TNFα) antagonists infliximab and adalimumab are effective therapies for patients with moderate-to-severe UC.[6–8] Golimumab is a fully human monoclonal anti-TNFα antibody[9] approved for monthly subcutaneous (SC) treatment of rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis.[10–15]

The target population for the golimumab UC development program was anti-TNFα biologic-naive adult patients with moderate-to-severe UC who had an inadequate response to or failed to tolerate conventional oral therapies. In this program, patients received SC or intravenous (IV) golimumab induction therapy (Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment [PURSUIT]-SC; ClinicalTrials.gov number: NCT00487539; PURSUIT-IV; ClinicalTrials.gov number: NCT00488774). Patients who completed an induction study were eligible to participate in this maintenance study (PURSUIT-Maintenance [PURSUIT-M]) to evaluate SC golimumab maintenance therapy administered every 4 weeks through week 52.

Materials and Methods

Patients

PURSUIT-M (NCT00488631) was a phase 3, multicenter, placebo-controlled, double-blind, randomized-withdrawal study conducted at 251 centers between September 2007 and October 2011. The institutional review board or ethics committee at each site approved the protocol, and patients provided written informed consent. All authors had access to the study data and reviewed and approved the final manuscript.

Participants in PURSUIT-M had completed 1 of 2 golimumab induction studies: PURSUIT-IV or PURSUIT-SC (Figure 1). Patients eligible for PURSUIT-IV or PURSUIT-SC had an established diagnosis of UC with moderate-to-severe disease activity, defined as a Mayo score of 6–12, with an endoscopic subscore of 2 or more.[6,16,17] Patients with isolated proctitis were excluded from the induction studies. Patients had an inadequate response to or had failed to tolerate 1 or more of the following conventional therapies: oral 5-aminosalicylates (5-ASAs), oral corticosteroids, immunosuppressives (azathioprine or 6-mercaptopurine); or were corticosteroid-dependent (ie, could not taper corticosteroids without recurrence of UC symptoms). Patients receiving 5-ASAs or immunosuppressives at baseline of the PURSUIT-IV or PURSUIT-SC studies were required to have maintained stable doses throughout induction and maintenance. Patients receiving corticosteroids at baseline of the PURSUIT-IV or PURSUIT-SC studies had to have maintained doses throughout induction. After induction, patients in clinical response and receiving concomitant corticosteroids at baseline of the PURSUIT-M study were required to taper corticosteroids (for doses of >20 mg/day prednisone or equivalent: taper daily dose by 5 mg/wk; for dose of ≤20 mg/day prednisone or equivalent: taper daily dose by 2.5 mg/wk) beginning at baseline. Additional clinical and concomitant medication eligibility criteria for induction study participation have been described previously.[18]

Patients were screened for tuberculosis before entry into one of the companion induction trials; those with active or previously identified latent disease were excluded. Patients with newly identified latent disease had to initiate appropriate treatment before being enrolled into and beginning treatment in this study. During the trials, patients were evaluated routinely for signs and symptoms of active tuberculosis (Supplementary Appendix).

Study Design

Patients who responded to golimumab induction therapy (n = 464) were randomized at the baseline visit in a 1:1:1 ratio to receive SC placebo, golimumab (Simponi; Janssen Biotech, Inc, Horsham, PA) 50 mg, or golimumab 100 mg every 4 weeks through week 52 (Figure 1). Treatment allocation used an adaptive randomization procedure based on 3 factors: (1) investigative site, (2) a 4-level cross-classification of clinical remission status and corticosteroid use at PURSUIT-M baseline, and (3) previous induction therapy (IV golimumab 1 mg/kg, 2 mg/kg, or 4 mg/kg; SC golimumab 100/50 mg, 200/100 mg, or 400/200 mg).

Placebo-induction responders (Supplementary Appendix), and placebo- or golimumab-induction nonresponders, also were eligible, but were not randomized. Placebo-induction responders (n = 129) received placebo every 4 weeks through week 52. Golimumab-induction (n = 405) or placebo-induction (n = 230) nonresponders received golimumab 100 mg every 4 weeks through week 12 (Figure 1), were assessed at week 16, and patients were discontinued from the study if disease activity was not improved.

Induction-therapy responders who subsequently lost clinical response could have their treatment modified as follows: placebo-treated patients received golimumab 100 mg every 4 weeks, patients treated with golimumab 50 mg were re-randomized to receive golimumab 50 mg or 100 mg every 4 weeks, and patients treated with golimumab 100 mg initially were re-randomized to receive golimumab 100 mg or 200 mg every 4 weeks. After a protocol amendment, dose adjustment to 200 mg every 4 weeks was discontinued; patients initially randomized to 100 mg continued to receive 100 mg, and patients who already had their dose increased to golimumab 200 mg were decreased to golimumab 100 mg (Supplementary Figure S1).

Study Evaluations

Clinical efficacy was assessed with the Mayo score, a composite activity index calculated as the sum of stool frequency relative to normal, rectal bleeding, endoscopic findings, and physician's global assessment subscores.[16] Mayo scores, ranging from 0 to 12 (higher scores indicate more severe disease), were calculated at weeks 0, 30, and 54. Partial Mayo scores (ie, Mayo score excluding the endoscopy subscore with values ranging from 0 to 9) were calculated at all other visits.

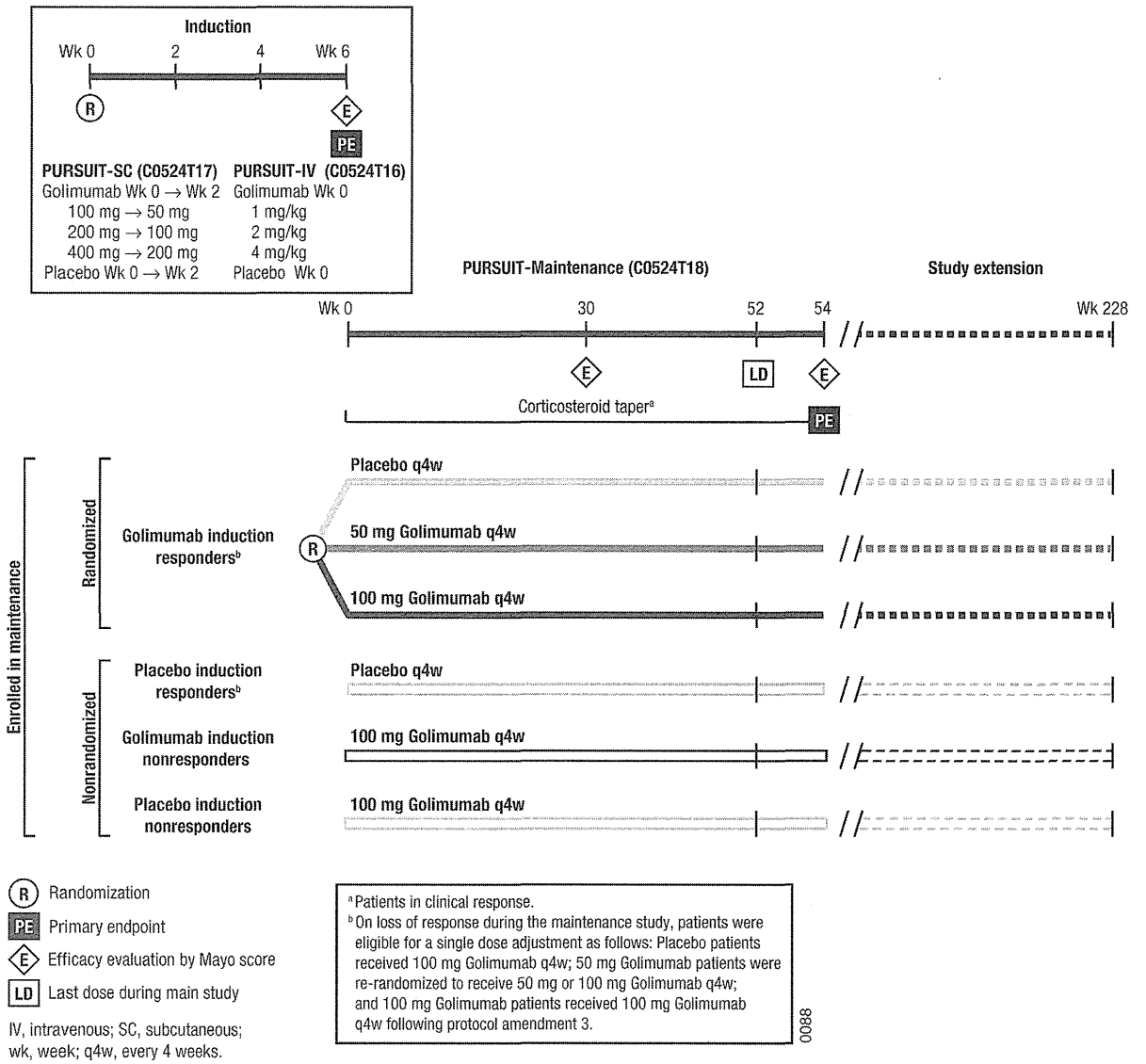


Figure 1. PURSUIT-M study design.

Patients experiencing a clinical flare (ie, increased disease activity) at any time during the study were required to undergo sigmoidoscopy for calculation of the Mayo score and assessment for loss of clinical response (ie, no longer in clinical response by Mayo score criteria). A clinical flare was defined as either an increase from PURSUIT-M baseline in the partial Mayo score of 2 or more points with an absolute partial Mayo score of 4 or higher, or an absolute partial Mayo score of 7 or more points.

Serum samples were collected for determination of golimumab concentrations (weeks 30 and 54, including trough concentrations at weeks 0, 4, 8, 12, 20, 28, 36, and 44) using a validated electrochemiluminescent assay (lowest quantifiable concentration in a sample was 0.039 μg/mL),[19] and identification of antibodies to golimumab (weeks 30 and 54) using a validated antigen bridging immunoassay.[20]

Adverse events and concomitant medications were recorded through week 54.

Statistical Analyses

For all efficacy analyses, patients were analyzed according to the assigned treatment group regardless of the actual treatment received. Unless indicated otherwise, efficacy analyses were based on the primary analysis population (Supplemental Consort Diagram), defined as patients randomized at PURSUIT-M baseline (ie, patients showing clinical response to golimumab induction per the interactive voice response system). Nonrandomized patients were included in demographic, pharmacokinetic, and safety summaries only. All patients who received at least one maintenance dose of study agent were included in pharmacokinetic and safety analyses; safety

summaries were based on actual treatment received (Supplemental Consort Diagram).

The primary analysis population included 456 of 464 randomized golimumab-induction responders; eight patients (1.7%) were excluded from the primary analysis population because of noncompliance with good clinical practice at three sites (two from the PURSUIT-SC and one from the PURSUIT-IV companion induction studies). Efficacy data for one of these eight patients were excluded prospectively. The remaining 7 randomized patients were retrospectively excluded following identification of noncompliance after acceptance of the original manuscript for publication in this journal. Further details are described in the Supplemental Appendix. With the exclusion of efficacy data from this additional site, the efficacy results in this final version differ from the originally accepted version published online. Data from patients at these three sites were included in demography, pharmacokinetic, and safety summaries.

Clinical response was defined as a decrease from the baseline value (observed in the preceding induction study) in the Mayo score by 30% or more and 3 or more points, with either a decrease in the rectal bleeding subscore of 1 or more or a rectal bleeding subscore of 0/1.[6,17] Clinical remission was defined as a Mayo score of 2 or fewer points, with no individual subscore greater than 1.[6,17] Mucosal healing was defined as a Mayo endoscopy subscore of 0/1.[6,17]

Primary end point. The primary end point was maintenance of clinical response through week 54 among golimumab-induction responders. Patients were assessed for UC disease activity using the Mayo score at weeks 30 and 54 and by partial Mayo score every 4 weeks (loss of response was confirmed by endoscopy) to substantiate that patients had maintained clinical response at each visit. Therefore, patients who maintained clinical response were considered to be in a state of continuous clinical response through week 54.

Proportions of patients in clinical response through week 54 were compared between each golimumab dose and placebo using the Cochran–Mantel–Haenszel chi-square test stratified by clinical remission status at PURSUIT-M baseline and induction dose (IV golimumab 1, 2, or 4 mg/kg; SC golimumab 100/50, 200/100, or 400/200 mg). A fixed-sequence testing procedure (ie, testing golimumab 100 mg vs placebo first, then, if positive, testing 50 mg vs placebo) was used to control the overall type I error rate at the 0.05 level. This study was considered positive if the golimumab 100 mg group clinical response rate was significantly greater than that for placebo. Assuming a 35% clinical response rate for placebo and 55% for golimumab 100 mg, approximately 128 patients in each randomized group (384 patients overall) provided an overall power of 90% for the primary end point at a 0.05 significance level based on a 2-sided chi-square test.

Major secondary and other efficacy end points. The analysis population for major secondary end points was golimumab-induction responders. Prespecified major secondary end points were as follows: (1) clinical remission at both weeks 30 and 54; (2) mucosal healing at both weeks 30 and 54; (3) clinical remission at both weeks 30 and 54 among patients who had clinical remission at PURSUIT-M baseline; and (4) corticosteroid-free clinical remission at week 54 among patients receiving concomitant corticosteroids at PURSUIT-M baseline.

If the test comparing the 100-mg golimumab and placebo groups was positive for the primary end point, the significance of the major secondary end points was to be tested. To control the family-wise type 1 error rate at the 0.05 (2-sided) significance level, the fixed sequence testing procedure described for the primary end point was applied within each major secondary end point. Major secondary end points were tested in a hierarchical manner in the order listed earlier. For a given major secondary end point, testing was performed for the 100-mg golimumab group if the test for the preceding end point was positive for the 100-mg group whether or not the 50-mg group was positive for the preceding end point. Likewise, the 50-mg group was tested if the 100-mg group tested positive for the same major secondary end point and the 50-mg group was positive for the preceding end point.

All other secondary end points were not controlled for multiplicity. Nominal *P* values are provided.

Treatment failure rules were applied to all efficacy end points unless otherwise specified. Patients who had (1) protocol-prohibited concomitant UC medication change(s), (2) a colectomy (partial or total) or an ostomy, (3) discontinued study agent because of a lack of therapeutic effect, or (4) a dose adjustment before week 54 were considered to be a treatment failure from the time of the event forward. For dichotomous end points, patients deemed a treatment failure before the designated analysis time point were considered not to have achieved the respective end point. For continuous end points (except for corticosteroid-based end points), patients designated treatment failures had their induction baseline value carried forward from the time of treatment failure. For corticosteroid-based end points, the PURSUIT-M baseline value was carried forward. Treatment failure rules overrode other data-handling rules.

Patients with missing data for a dichotomous end point were considered as not having achieved the end point. For continuous end points, generally, the last observation in PURSUIT-M was carried forward for patients with missing data. However, patients with missing Mayo and partial Mayo subscores had their last available PURSUIT-M subscores carried forward.

Analyses to identify predictors of clinical remission at both weeks 30 and 54 were performed post hoc based on a logistic regression model using a stepwise selection method to identify covariates from a pool of induction baseline demographic and disease characteristics, clinical laboratory values, and concomitant UC medication use (a significance level of 0.25 was required to allow a variable into the model, and a significance level of 0.10 was required for a variable to stay in the model).

Safety was evaluated in all randomized patients across the placebo, golimumab 50-mg, and golimumab 100-mg groups to provide a balanced comparison of treatments and all treated patients, including both randomized and nonrandomized patients, to provide an overall safety summary. A Cox proportional hazards model including covariates for treatment group, baseline corticosteroid use (yes vs no), and baseline immunomodulator use (yes vs no) assessed the impact of UC therapies on infections.

Results

Patient Disposition, Baseline Characteristics, and Baseline Concomitant Medications

The PURSUIT-M study was conducted at 251 sites in Eastern Europe (477 patients), North America (323

patients), Asia Pacific and South Africa (237 patients), and Western Europe and Israel (191 patients). Overall, 1228 patients were enrolled from the induction studies, including 464 patients who were randomized in the primary analysis population (ie, were in clinical response to golimumab at week 6 of an induction study) and 764 patients who were enrolled but not randomized (ie, nonrandomized patients).

Induction study baseline demographic characteristics and concomitant medication use were similar among randomized treatment groups at PURSUIT-M baseline (Table 1). Almost half of the randomized patients were receiving corticosteroids, with approximately 35% receiving 20 mg or more of prednisone equivalent/day. The median baseline (week 0 PURSUIT-M) dose of corticosteroids was 20 mg prednisone equivalent per day. Approximately 30% of patients were receiving azathioprine/6-mercaptopurine, and 80% were receiving 5-ASAs.

Induction study baseline characteristics of the nonrandomized patients generally were consistent with those of randomized patients at PURSUIT-M baseline. The proportion of males, the proportion of patients with severe disease, and median C-reactive protein concentrations were higher for golimumab-induction nonresponders (65.9%, 13.1%, and 6.3 mg/L, respectively) than for randomized golimumab-induction responders (51.9%, 6.7%, and 3.6 mg/L, respectively) (Table 1).

Among randomized patients, 75.6% (351 of 464) of patients completed the study through week 54; more than 70% of patients completed the study in each group (Figure 2). Among nonrandomized patients, 55.9% (427 of 764) of patients completed the study through week 54; 73.6% (95 of 129) of placebo-induction responders, and 57.0% (131 of 230) and 49.6% (201 of 405) of placebo and golimumab-induction nonresponders, respectively, completed the study through week 54.

Efficacy

Primary end point. Among the primary analysis population (N = 456), the proportions of patients who maintained a clinical response through week 54 were significantly greater in the 100-mg and 50-mg groups (49.7% and 47.0%, respectively) compared with the placebo group (31.2%; $P < .001$ and $P = .010$, respectively; Figure 3*A*). The numbers needed to treat (NNT) for clinical response through week 54 were 5 and 6 for the 100- and 50-mg golimumab groups, respectively.

Consistency of effect. The treatment effect of golimumab 100 mg and 50 mg generally was consistent across subgroups of patients (Supplementary Figures S2 and S3), including study center location. The inconsistency of efficacy results among induction baseline corticosteroid subgroups (none, <20 mg/kg, ≥20 mg/day, ≥30 mg/day, and ≥40 mg/day) made it difficult to draw definitive conclusions regarding any corticosteroid carryover effects (Supplementary Appendix).

Major secondary end points. Among golimumab-induction responders, the proportion of patients in clinical remission at both weeks 30 and 54 was significantly greater for golimumab 100 mg (27.8%) compared with placebo (15.6%; $P = .004$). The corresponding value for the golimumab 50-mg group (23.2%) was greater than that for placebo; however, the difference was not statistically significant (Figure 3*B*). According to the prespecified testing procedure, because the 50-mg group did not test positive for this end point, statistical significance for the 50-mg group for subsequent major secondary end points cannot be claimed as significant. The NNT for patients in remission at both weeks 30 and 54 were 8 and 13 for the 100- and 50-mg golimumab groups, respectively.

In a post hoc logistic regression analysis, lower baseline Mayo score, lower baseline fecal lactoferrin level, and higher baseline albumin level were associated with greater proportions of patients achieving clinical remission at both weeks 30 and 54 (Supplementary Table S1).

The proportion of patients with mucosal healing at both weeks 30 and 54 was significantly greater for patients receiving golimumab 100 mg (42.4%) compared with placebo (26.6%; $P = .002$). The mucosal healing rate for patients receiving golimumab 50 mg was 41.7% (Figure 3*C*).

Approximately 35% (160 of 456) of patients were in clinical remission at the baseline visit of PURSUIT-M. Numerically greater proportions of patients who received 100-mg or 50-mg golimumab maintained clinical remission (38.9% and 36.5%, respectively) than those assigned to placebo (24.1%); however, the differences were not statistically significant (Figure 3*D*).

Approximately 54% of patients were receiving concomitant corticosteroids at baseline, of whom the proportions of patients in corticosteroid-free clinical remission at week 54 were 23.2%, 28.2%, and 18.4% in the 100-mg, 50-mg, and placebo groups, respectively (Figure 3*E*). The NNT for patients achieving corticosteroid-free clinical remission at week 54 were 21 and 10 for the 100- and 50-mg golimumab groups, respectively.

Additional secondary end points. Reductions in median partial Mayo scores observed at baseline of PURSUIT-M among golimumab-induction responders (ie, decrease of 4 points from induction baseline; Figure 4*A*) were maintained in the 100-mg and 50-mg groups through weeks 52 and 48, respectively (with small increases at weeks 54 and 52, respectively), whereas the median partial Mayo score in the placebo group increased after week 8 and increased to a value approaching that at induction baseline at week 54 (nominal $P < .001$ and $P = .002$, respectively). In addition, time to loss of clinical response among golimumab-induction responders was longer in the 100-mg and 50-mg groups (>54 weeks for both) compared with placebo (27 weeks; nominal $P < .001$ and $P = .003$, respectively) (Figure 4*B*).

Greater proportions of golimumab-treated patients achieved clinical remission at week 30 (nominal significance for both dose groups) and week 54 (nominally significant for the 100-mg group only) compared with those in the placebo group (Table 2). Among golimumab-induction responders in clinical remission at week 0, a post hoc analysis showed longer time to loss of clinical remission in patients

Table 1. Demographics, Disease Characteristics, and Concomitant Medications

Characteristic	Randomized patients				Nonrandomized patients			Randomized and nonrandomized patients total (N = 1228)
		Golimumab				Golimumab 100 mg		
	Placebo[a] (N = 156)	50 mg (N = 154)	100 mg (N = 154)	Total (N = 464)	Placebo[b] (N = 129)	Placebo nonresponders (induction) (N = 230)	Golimumab nonresponders (induction) (N = 405)	
Sex, n (%)								
Male	75 (48.1)	77 (50.0)	89 (57.8)	241 (51.9)	61 (47.3)	131 (57.0)	267 (65.9)	700 (57.0)
Female	81 (51.9)	77 (50.0)	65 (42.2)	223 (48.1)	68 (52.7)	99 (43.0)	138 (34.1)	528 (43.0)
Race, n (%)								
Caucasian	137 (87.8)	138 (89.6)	130 (84.4)	405 (87.3)	106 (82.2)	181 (78.7)	320 (79.0)	1012 (82.4)
Black	1 (0.6)	2 (1.3)	5 (3.2)	8 (1.7)	6 (4.7)	4 (1.7)	7 (1.7)	25 (2.0)
Asian	12 (7.7)	12 (7.8)	14 (9.1)	38 (8.2)	15 (11.6)	37 (16.1)	61 (15.1)	151 (12.3)
Other	6 (3.8)	2 (1.3)	5 (3.2)	13 (2.8)	2 (1.6)	8 (3.5)	17 (4.2)	40 (3.3)
Age, y								
Mean ± SD	40.2 ± 14.05	41.4 ± 13.84	39.1 ± 13.11	40.2 ± 13.68	38.0 ± 13.27	40.3 ± 12.67	41.2 ± 13.60	40.3 ± 13.44
Disease duration, y								
Mean ± SD	6.9 ± 6.96	6.8 ± 6.93	7.2 ± 7.04	7.0 ± 6.96	6.3 ± 7.02	6.2 ± 6.40	6.1 ± 5.94	6.5 ± 6.54
Median	4.2	4.5	4.8	4.6	3.8	4.3	4.3	4.3
Mayo score (0–12)								
Mean ± SD	8.3 ± 1.37	8.1 ± 1.38	8.5 ± 1.34	8.3 ± 1.36	8.2 ± 1.65	8.2 ± 1.42	8.6 ± 1.54	8.4 ± 1.47
Median	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Severity of UC disease, n (%)								
n	156	154	154	464	129	229	405	1227
Moderate disease (Mayo score ≥6 to ≤10)	145 (92.9)	145 (94.2)	143 (92.9)	433 (93.3)	116 (89.9)	217 (94.8)	352 (86.9)	1118 (91.1)
Severe disease (Mayo score >10)	11 (7.1)	9 (5.8)	11 (7.1)	31 (6.7)	13 (10.1)	12 (5.2)	53 (13.1)	109 (8.9)
CRP, mg/L								
n	150	149	152	451	122	225	399	1197
Mean ± SD	9.6 ± 15.48	8.5 ± 12.79	8.9 ± 14.74	9.0 ± 14.36	9.5 ± 13.72	9.6 ± 14.78	13.2 ± 20.04	10.6 ± 16.57
Median	3.2	4.5	3.4	3.6	3.7	4.7	6.3	4.6
Any UC medication, n (%)	148 (94.9)	144 (93.5)	143 (92.9)	435 (93.8)	122 (94.6)	213 (92.6)	377 (93.1)	1147 (93.4)
Corticosteroid[c]	83 (53.2)	77 (50.0)	79 (51.3)	239 (51.5)	63 (48.8)	92 (40.0)	168 (41.5)	562 (45.8)
≥20 mg/day PEq	59 (37.8)	52 (33.8)	55 (35.7)	166 (35.8)	44 (34.1)	44 (19.1)	105 (25.9)	359 (29.2)
<20 mg/day PEq	24 (15.4)	25 (16.2)	24 (15.6)	73 (15.7)	19 (14.7)	48 (20.9)	63 (15.6)	203 (16.5)
Budesonide	5 (3.2)	6 (3.9)	4 (2.6)	15 (3.2)	1 (0.8)	7 (3.0)	9 (2.2)	32 (2.6)
Immunomodulatory drugs	52 (33.3)	47 (30.5)	48 (31.2)	147 (31.7)	37 (28.7)	79 (34.3)	131 (32.3)	394 (32.1)
6-MP/AZA	51 (32.7)	45 (29.2)	48 (31.2)	144 (31.0)	37 (28.7)	72 (31.3)	125 (30.9)	378 (30.8)
Methotrexate	1 (0.6)	2 (1.3)	0 (0.0)	3 (0.6)	0 (0.0)	7 (3.0)	6 (1.5)	16 (1.3)
Aminosalicylates	125 (80.1)	128 (83.1)	119 (77.3)	372 (80.2)	115 (89.1)	183 (79.6)	340 (84.0)	1010 (82.2)

NOTE. Data from week 0 of induction studies for enrolled patients.
6-MP, 6-mercaptopurine; AZA, azathioprine; CRP, C-reactive protein; PEq, prednisone equivalent.
[a]Patients who were in clinical response to golimumab induction dosing and were randomized to placebo on entry into this maintenance trial.
[b]Patients who were in clinical response to placebo induction dosing and received placebo on entry into this maintenance trial.
[c]Excluding budesonide.

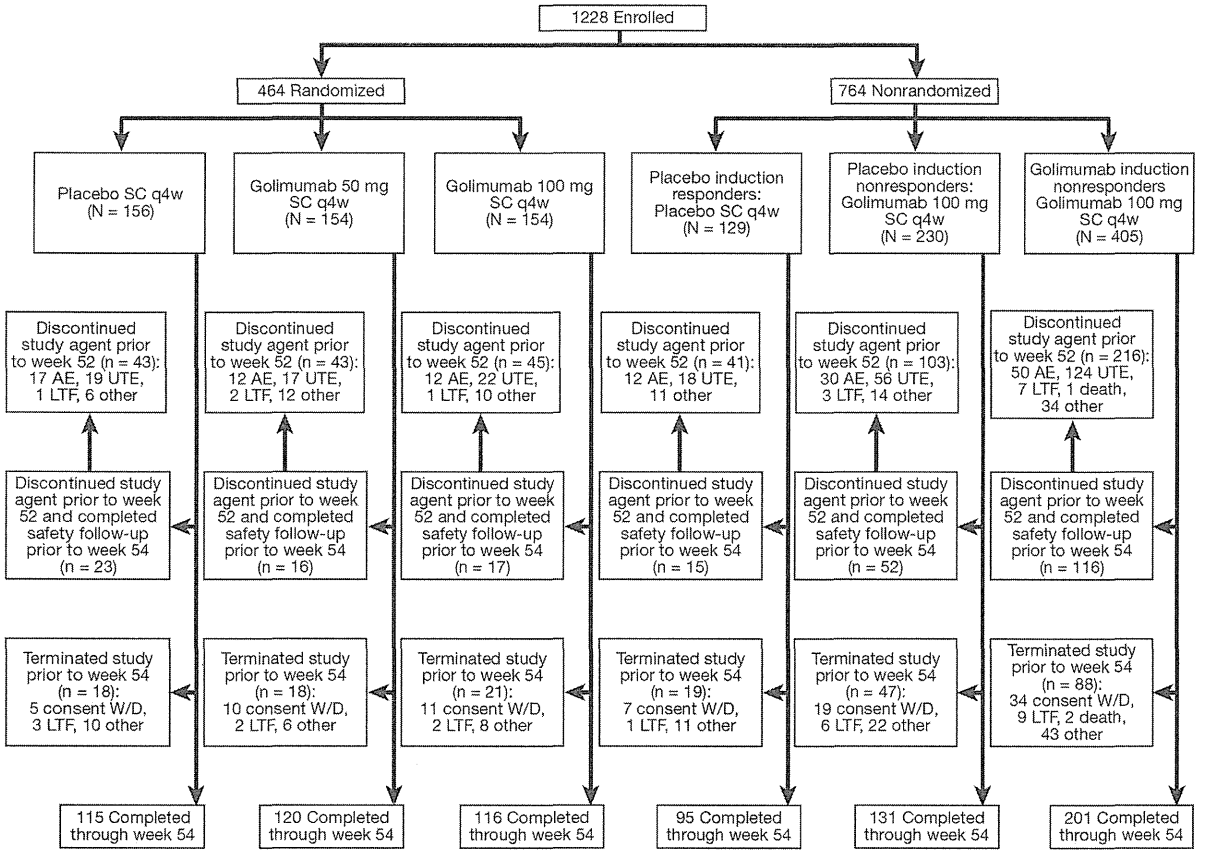


Figure 2. Study participation status through week 54. AE, adverse event; LTF, lost to follow-up evaluation; q4w, every 4 weeks; UTE, unsatisfactory therapeutic effect; W/D, withdrawn.

who received golimumab 100 mg (50 weeks) or 50 mg (52 weeks) compared with placebo (27 weeks; nominal $P = .017$ and $P = .207$, respectively) (Figure 4*C*).

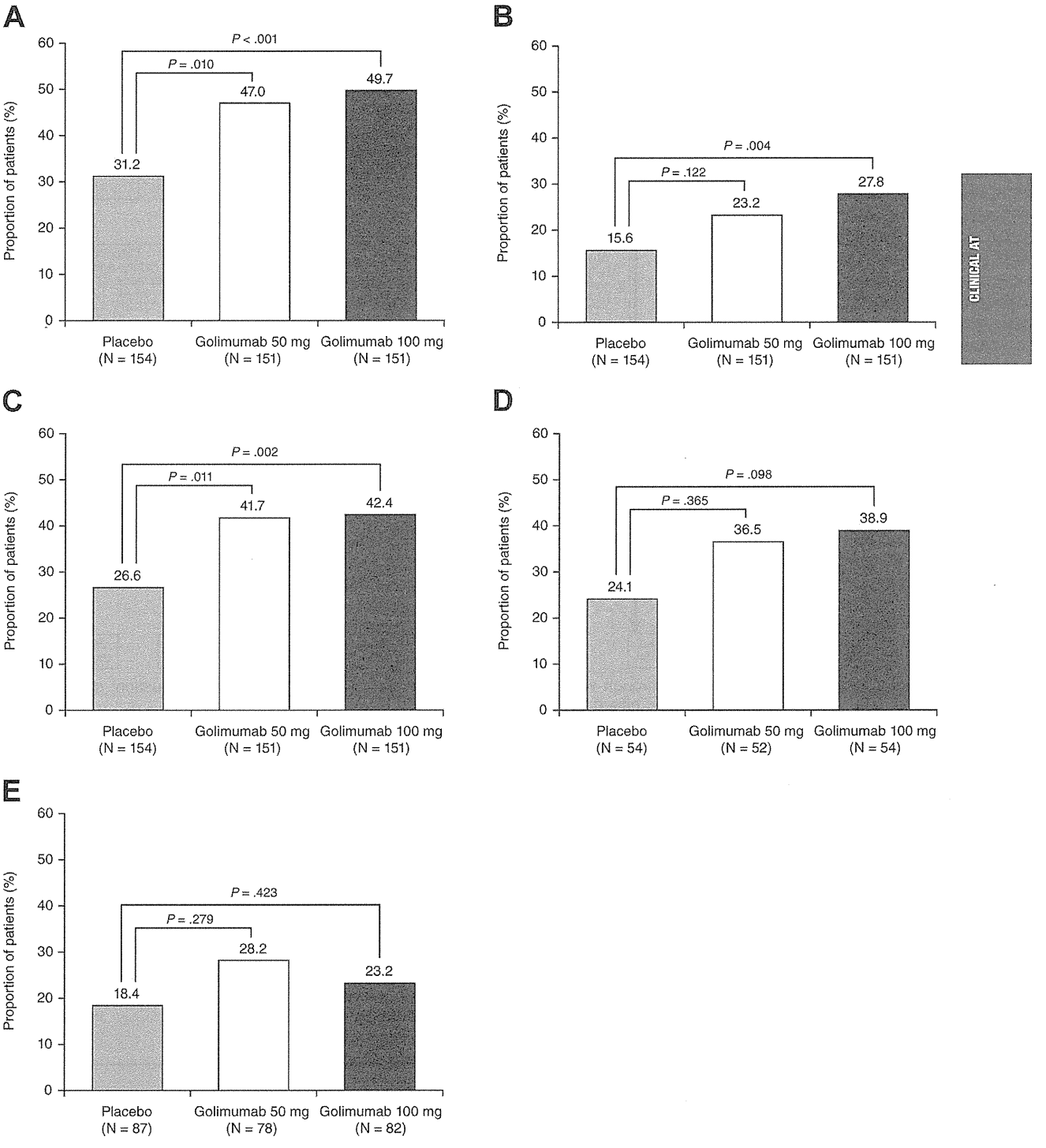
Among golimumab-induction responders receiving corticosteroids at baseline, a greater proportion of patients in the 100-mg group (38.5%) and a greater proportion of patients in the 50-mg group (38.5%; nominal $P = .026$) maintained clinical response through week 54 and were corticosteroid-free at week 54 compared with the placebo group (20.7%) (Table 2).

At week 54, the proportions of patients with normal or inactive mucosal disease (ie, an endoscopy subscore = 0) were greater in the 100-mg (21.9%) and 50-mg (25.8%) groups compared with the placebo group (13.0%; nominal $P = .033$ and .011, respectively).

Dose adjustment. One hundred sixty-nine (37.1%) patients in the primary analysis population had a dose adjustment, of whom 75 (48.7%), 43 (28.5%), and 51 (33.8%) received placebo, 100-mg golimumab, or 50-mg golimumab at baseline, respectively.

The efficacy of dose adjustment was compared in the 50-mg group between patients who continued the 50-mg dose and those who increased to the 100-mg dose because this was the only dose-adjustment strategy with a

Figure 3. Proportion of golimumab-induction responders who (*A*) maintained clinical response through week 54; (*B*) achieved clinical remission at both weeks 30 and 54; (*C*) achieved mucosal healing at both weeks 30 and 54; (*D*) maintained clinical remission among those who were in clinical remission at baseline; and (*E*) achieved corticosteroid-free clinical remission at week 54 among those who were receiving corticosteroids at baseline. (*A–E*) Patients who had a prohibited change in UC medication, an ostomy or colectomy, a dose adjustment, or discontinued study agent because of a lack of therapeutic effect before the week 54 visit were considered not to have a clinical response, be in clinical remission, be in corticosteroid-free clinical remission, or have mucosal healing. (*A–D*) Patients who had all 4 Mayo subscores missing at weeks 30 or 54 were considered not to be in clinical response or clinical remission. Patients who had a missing endoscopy subscore at week 30 or week 54 were considered not to have mucosal healing. (*E*) Patients who had a missing value in corticosteroid use at a time point had their last available value carried forward to that time point. UC, ulcerative colitis.



comparator. Among the 51 patients who received 50-mg golimumab, 26 patients continued receiving 50 mg and 25 patients increased their dose to golimumab 100 mg upon loss of response. Clinical response at week 54 for patients who increased to 100-mg golimumab did not differ from that observed in patients who continued the 50-mg dose (28.0% vs 34.6%).

Among those patients who had an endoscopy subscore at the time of dose adjustment (Supplementary Table S2), approximately 90% presented with endoscopy subscores of

2 or 3 at the time of dose adjustment and therefore did not meet the criteria of mucosal healing (endoscopy subscore of 0 or 1).

Serum golimumab concentrations. Before administration of the baseline maintenance dose, median serum golimumab concentrations were similar among patients in the 3 randomized maintenance treatment groups: 2.46, 2.28, and 2.50 μg/mL for the placebo, 50-mg, and 100-mg golimumab groups, respectively. Median serum trough golimumab concentrations were stable from weeks 8 through 44 in the golimumab 50-mg (0.69–0.83 μg/mL) and 100-mg (1.33–1.58 μg/mL) groups. These results suggest that a steady-state was reached approximately 8 weeks after patients began receiving golimumab maintenance doses. Serum golimumab concentrations at week 8 in randomized patients from the IV induction treatment groups were similar to those in patients randomized to the same maintenance dose from the SC induction treatment groups. For example, among patients randomized to SC golimumab 100 mg, serum golimumab concentrations at week 8 in randomized patients from the IV induction treatment groups ranged from 1.17 to 1.59 μg/mL compared with a range from 1.14 to 1.60 μg/mL in randomized patients from the SC induction treatment groups.

In general, from week 8 through week 54, patients in the 100-mg golimumab group had serum golimumab concentrations that were approximately twice those of patients in the 50-mg golimumab group. At weeks 30 and 54, when key efficacy end points were assessed, median serum golimumab concentrations were 1.73 μg/mL and 1.81 μg/mL, respectively, in the golimumab 50-mg group, and 3.81 μg/mL and 3.52 μg/mL, respectively, in the golimumab 100-mg group.

Among randomized patients in the combined golimumab 50-mg and 100-mg groups, greater proportions of patients in the higher serum golimumab concentration quartiles achieved clinical response through week 54 or clinical remission at both weeks 30 and 54 when compared with those in the lower serum concentration quartiles (Figure 4*D*). Efficacy in patients who received either IV or SC induction therapy generally was similar (Supplementary Table S3).

Antibodies to golimumab. Among patients (including both randomized and nonrandomized) who had appropriate samples for analysis, the incidence of antibodies to golimumab through week 54 was 2.9% (32 of 1103 patients). The majority (21 of 32) had titers less than 1:640. Among patients with antibodies to golimumab, 31 were evaluable for neutralizing antibody assessment; of whom 21 (67.7%) tested positive.

The proportion of patients who were positive for antibodies to golimumab among those who were receiving concomitant immunomodulators was lower (1.1% [4 of 362]) compared with patients who were not receiving concomitant immunomodulators (3.8% [28 of 741]; nominal $P = .013$) (Supplementary Table S4).

Safety

Among 464 randomized golimumab induction-responders, 66.0%, 72.7%, and 73.4% of patients in the placebo, golimumab 50-mg, and golimumab 100-mg groups, respectively, reported 1 or more treatment-emergent adverse event(s) (Table 3). With adjustment for length of follow-up evaluation, the incidences of adverse events per 100 patient-years were similar across treatment groups (Supplementary Table S5). Infections were reported in 28.2%, 39.0%, and 39.0%; serious adverse events were reported in 7.7%, 8.4%, and 14.3%; and serious infections were reported in 1.9%, 3.2%, and 3.2% of patients receiving placebo, golimumab 50 mg, and golimumab 100 mg, respectively. The proportion of patients who discontinued the study agent because of an adverse event through week 54 were 6.4%, 5.2%, and 9.1%, respectively. Injection-site reactions occurred in 1.9%, 1.9%, and 7.1% of patients in the placebo, golimumab 50-mg, and golimumab 100-mg groups, respectively. No injection-site reaction was serious, and no anaphylactic reactions were reported. Similar types of adverse events were observed among patients who had their golimumab dose increased, and no adverse events of interest occurred in patients who received golimumab 200 mg (Table 3).

Adverse events observed for all treated patients (Supplementary Table S6) were consistent with those observed for randomized patients. Overall, 63.9%, 72.7%, and 73.9% of patients who received placebo, golimumab 50 mg, or golimumab 100 mg experienced one or more adverse events.

Through week 54, there were 3 deaths reported, all in the 100-mg golimumab maintenance group. Causes of death were malnutrition and sepsis (golimumab 2 mg/kg IV induction), cardiac failure in a patient with a history of thrombosis (golimumab 400/200 mg SC induction), and disseminated tuberculosis in a patient who tested positive for latent tuberculosis on induction study entry (golimumab 200/100 mg SC induction) and was receiving isoniazid at the time of the event.

Six deaths were reported after week 54: pneumonia and heart failure (placebo SC induction and maintenance); biventricular heart dysfunction in the presence of pronounced atherosclerosis and stenosis affecting the aorta, large arteries, and coronary arteries (golimumab SC 100/50 mg induction, 50 mg maintenance); myocardial infarction in a patient with a history of myocardial infarction (placebo SC induction, 100 mg maintenance); gallbladder adenocarcinoma with liver metastasis (golimumab 2 mg/kg IV induction, 100 mg maintenance); sepsis (golimumab 2 mg/kg IV induction, 100 mg maintenance); and accidental nitrous oxide overdose (200/100 mg SC induction, 100 mg maintenance).

Tuberculosis was reported for 4 patients through week 54 (1 placebo maintenance [golimumab 4 mg/kg IV induction] and 3 golimumab 100 mg maintenance [1 each golimumab 400/200 mg SC, 4 mg/kg IV, and 200/100 mg SC induction] treated patients), including the fatal case described previously. Three patients had active tuberculosis and 1 patient was diagnosed with latent infection. Each of these patients was receiving concurrent corticosteroid therapy at screening before entry into the preceding induction study; 3 of these patients remained on corticosteroids at

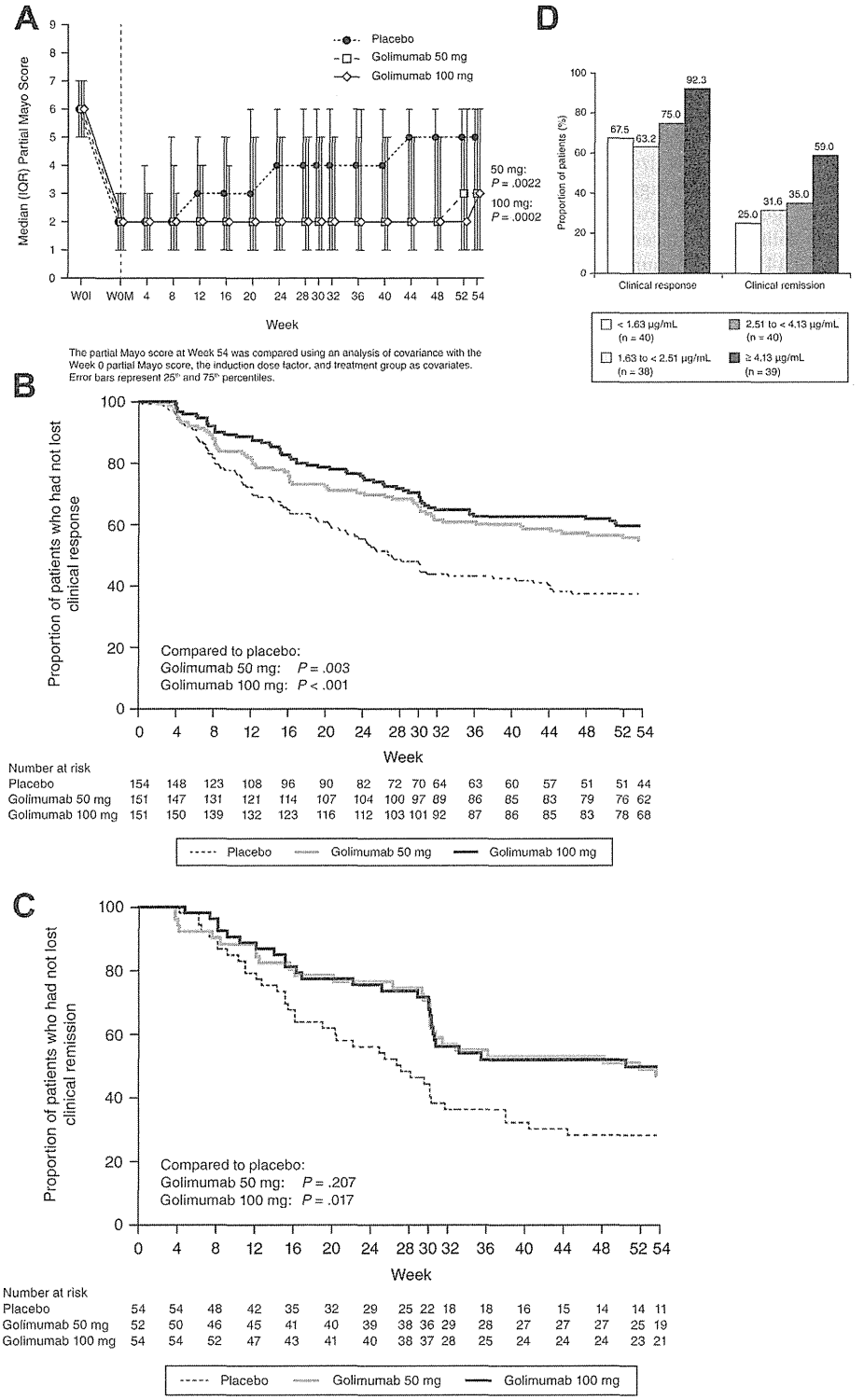


Figure 4. (*A*) Median (interquartile range [IQR]) partial Mayo score through week 54, (*B*) time to loss of clinical response, (*C*) time to loss of clinical remission among patients in clinical remission at baseline, (*D*) clinical response through week 54 or in clinical remission at both weeks 30 and 54 by serum golimumab concentration quartiles at week 54. W0I, week 0 of induction; W0M, week 0 of maintenance.

Table 2. Secondary Efficacy End Points

Variable	Placebo (N = 154)	Golimumab 50 mg (N = 151)	Golimumab 100 mg (N = 151)
Clinical remission,[ab] n (%)			
Week 30	35 (22.7)	54 (35.8)	60 (39.7)
P value		.013	<.001
Week 54	34 (22.1)	50 (33.1)	51 (33.8)
P value		.068	.011
Corticosteroid use[abc]			
Receiving corticosteroids at PURSUIT-M baseline, n	87	78	82
Maintained clinical response through week 54 and corticosteroid-free at week 54	18 (20.7)	30 (38.5)	25 (30.5)
P value		.026	.138

NOTE. Data are summarized for golimumab-induction responders.
[a]Patients who had a prohibited change in UC medication, an ostomy or colectomy, a dose adjustment, or discontinued study agent owing to a lack of therapeutic effect before the designated analysis time point are considered not to be in clinical remission and not to be corticosteroid free.
[b]Patients who had all 4 Mayo subscores missing at week 30 (or at week 54) are considered not to be in clinical remission at week 30 (or at week 54).
[c]Patients who had a missing value in corticosteroid use at week 54 had their last value carried forward.

the time of tuberculosis diagnosis. Another patient (golimumab 100 mg maintenance [golimumab 200/100 mg SC induction]) was diagnosed with a pleural effusion with a high adenosine deaminase concentration and subsequently was treated for suspicion of tuberculosis. Affected patients lived in endemic regions (ie, Poland, India, and South Africa).

Two patients developed serious opportunistic infections: 1 patient with cytomegalovirus infection identified as antigenemia in the blood with negative polymerase chain reaction on colon biopsy approximately 3 months after the last golimumab dose (golimumab SC 200/100 mg induction, placebo maintenance); and 1 patient with *Staphylococcus aureus* and *Nocardia* cultured from a brain abscess (golimumab SC 200/100 mg induction, golimumab 100 mg maintenance).

No association was observed between immunomodulator/corticosteroid use and infection development (Supplementary Table S7).

Three malignancies were reported through week 54 in patients receiving golimumab 100 mg maintenance; 2 of these (rectal cancer and thyroid cancer) presented with symptoms while the patients were receiving SC placebo induction and 1 (lung adenocarcinoma) occurred in a patient with a 40-year smoking history who received golimumab 200/100 mg SC induction therapy. Breast cancer was reported in a patient who had received only placebo during induction and maintenance.

Discussion

PURSUIT-M was a phase 3, placebo-controlled, randomized withdrawal study that evaluated the efficacy and safety of golimumab maintenance therapy. PURSUIT-M was a randomized withdrawal study of an anti-TNFα biologic agent in UC evaluating 2 golimumab maintenance doses. The target population for this study comprised patients with moderate-to-severe UC who were in clinical response to golimumab induction after participation in companion induction studies. The primary end point, maintenance of clinical response through week 54, was met with a study design providing rigor not previously applied. In earlier UC trials, clinical response was assessed at limited designated time points rather than throughout patients' study participation.[6,8] The study design of PURSUIT-M not only implemented the Mayo score to evaluate clinical response at weeks 30 and 54 but also used the partial Mayo score to substantiate that patients had maintained clinical response at each visit. Therefore, a patient who maintained clinical response was in a state of continuous response through the 54 weeks of the study. The 50-mg and 100-mg golimumab doses every 4 weeks were selected based on the efficacy and safety experience of these maintenance dosages from clinical trials in rheumatologic diseases.[10–15]

Maintenance therapy was evaluated in golimumab-induction responders. Significantly greater proportions of patients in the 50-mg and 100-mg groups (47.0% and 49.7%, respectively) maintained clinical response through week 54 compared with those in the placebo group (31.2%). Maintenance efficacy of 100-mg golimumab was confirmed further by the major secondary end points, for which the golimumab 100-mg group had significantly greater rates of clinical remission at both weeks 30 and 54 and mucosal healing at both weeks 30 and 54 when compared with the placebo group. The efficacy of golimumab was supported further by additional analyses of clinical response, clinical remission, and the partial Mayo score over time. For the prespecified and post hoc analyses related to maintenance of clinical remission among patients who were induced into clinical remission at week 6, there were positive but nonsignificant trends for the golimumab 100-mg group compared with placebo. It should be noted that the study lacked statistical power for these comparisons.