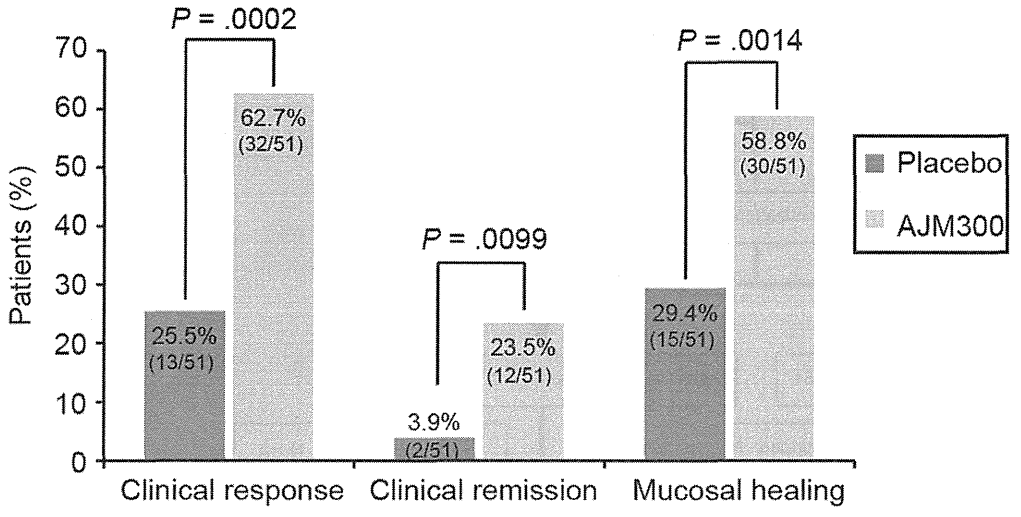

Figure 2. Proportion of patients with a clinical response, in clinical remission, and with mucosal healing at week 8. A clinical response was defined as a reduction in the Mayo Clinic score of at least 3 points and a decrease of at least 30% from the baseline score, with a decrease of at least 1 point on the rectal bleeding subscore or an absolute rectal bleeding subscore of 0 or 1. Clinical remission was defined as a Mayo Clinic score of 2 or lower and no subscore higher than 1. Mucosal healing was defined as an absolute endoscopic subscore of 0 or 1. The logistic regression model adjusted for the inadequate response or intolerance with mesalamine and/or corticosteroids, and the baseline Mayo Clinic score (6–7 or 8–10) was used for the statistical analysis of clinical response and mucosal healing. For the clinical remission, the logistic regression model adjusted for the baseline Mayo Clinic score (6–7 or 8–10) was used.

Patients receiving the active treatment had greater improvement in the partial Mayo Clinical score compared with patients receiving placebo (Figure 3). Each subscore (stool frequency, rectal bleeding, endoscopic findings, or physician's global assessment) of the Mayo Clinic score improved significantly in the active treatment group compared with that in the placebo group at week 8 (Supplementary Table 1). In addition, the endoscopic improvement was accompanied by a significant histologic improvement in the Riley score (Supplementary Table 2).

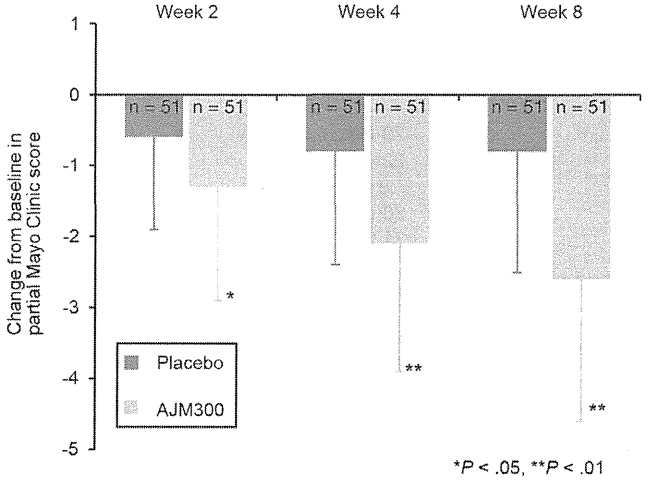

Figure 3. Change from baseline in the partial Mayo Clinic score. The partial Mayo Clinic score consists of the Mayo Clinic score minus the endoscopic subscore; ranging 0 to 9, with higher scores indicating more severe disease. The change from baseline in the partial Mayo Clinic score at each visit (weeks 2, 4, and 8) was indicated by the mean value with SD. *Asterisk* denotes significant difference between treatment groups at each visit (*t* test, $^{*}P < .05$; $^{**}P < .01$).

Pharmacodynamics

Anti-α4 integrin therapeutic agents are known to increase peripheral blood total lymphocyte counts. The significant increase in peripheral lymphocyte counts (as the pharmacodynamic action of AJM300) was observed at week 2 (mean, $2.7 \times 10^3/\mu L$ vs $1.8 \times 10^3/\mu L$ in the active treatment group and placebo group, respectively; difference, $0.8 \times 10^3/\mu L$; 95% CI: 0.5 to 1.2×10^3), as well as week 4 (mean, $2.7 \times 10^3/\mu L$ vs $1.8 \times 10^3/\mu L$; difference, $1.0 \times 10^3/\mu L$; 95% CI: 0.6 to 1.3×10^3) (Figure 4). At week 8, the day after last administration of the active treatment, the pharmacodynamic action disappeared (mean, $2.0 \times 10^3/\mu L$ vs $1.8 \times 10^3/\mu L$; difference, $0.2 \times 10^3/\mu L$; 95% CI: -0.1 to 0.5×10^3). As for the effect on leukocytes lacking α4 integrin, no change in neutrophil counts was observed throughout the study (data not shown).

Safety

Data for all randomized patients were used for the safety analysis. No important differences were observed between the treatment groups in the incidence of adverse events, 49.0% (25 of 51 patients) in the active treatment group and 56.9% (29 of 51 patients) in the placebo group (Table 2). The grades of all the adverse events were mild or moderate in severity, and no serious adverse events were observed in this study. The most common adverse events were nasopharyngitis and UC. The incidence of nasopharyngitis was similar between the treatment groups, 9.8% (5 of 51 patients) in the active treatment group and 7.8% (4 of 51

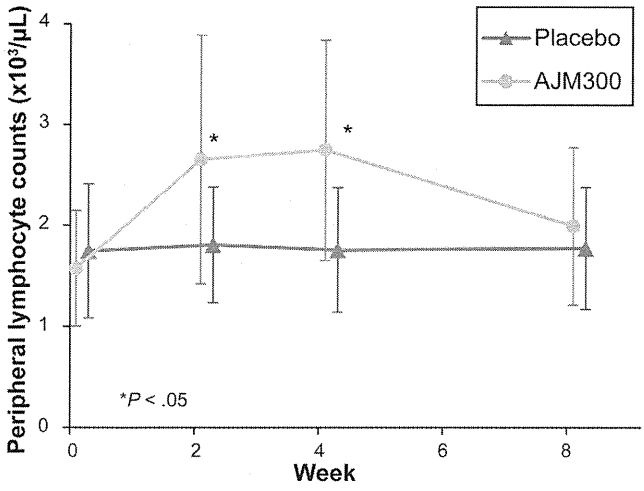

Figure 4. Peripheral lymphocyte counts. Peripheral lymphocyte counts of patients with active UC before and after receiving placebo or AJM300. Blood samples were obtained at weeks 0, 2, 4 and 8. Study drug treatment was terminated in the morning on the day before the last visit (week 8) to confirm the disappearance of the pharmacological activity of AJM300 at week 8. Bars are ±SD, and *asterisk* denotes significant difference between treatment groups at each evaluation time (*t* test, $P < .05$).

patients) in the placebo group. The adverse events related to UC were higher in the placebo group (17.6%; 9 of 51 patients) than those in the active treatment group (3.9%; 2 of 51 patients). Adverse events leading to discontinuation of the study drug included exacerbation of UC (1 patient in the active treatment group and 8 in the placebo group) and abnormality of liver function (1 patient in the placebo group). The incidence of drug-related adverse events was 21.6% (11 of 51 patients) in the active treatment group and 7.8% (4 of 51 patients) in the placebo group, all of which were mild. There were no frequent drug-related adverse events (>5.0%). Although higher incidences of drug-related adverse events, particularly abnormalities in the laboratory test values, were observed in the active treatment group than in the placebo group, these changes were mild and recovered without any treatment. Overall, infection, neurologic symptom, or onset of PML was not observed in this study.

Discussion

This is the first study to demonstrate the clinical benefit of an orally active small-molecule α4 integrin antagonist for UC. In this phase 2a study, AJM300 960 mg 3 times daily significantly improved clinical response and clinical remission at week 8 compared with placebo in patients with moderately active UC. Subgroup analysis showed the therapeutic benefit of the active treatment regardless of disease duration, extent of disease, and baseline Mayo Clinic scores. A higher proportion of patients achieved mucosal healing in the active treatment group compared with the placebo group. Recent evidence has emphasized the importance of mucosal healing as a treatment goal for UC.[22] It has been reported that the most favorable subsequent clinical outcomes were observed in patients with early achievement of endoscopic subscore of 0.[23] There was a trend toward an increase in the number of patients with endoscopic subscore of 0 in the active treatment group compared with the placebo group. Furthermore, a significantly better histologic improvement at week 8 was obtained in patients treated with the active treatment compared with those with placebo.

Rationales for the development of AJM300 were the achievements of the oral anti-α4 integrin therapy. Oral drug formulations often provide good drug adherence and may resolve some issues in biologic therapy. Adverse effects

Table 2. Summary of Adverse Events

	Overall safety population (n = 102), n (%)			
	Placebo (n = 51)		AJM300 (n = 51)	
Event	All	Study drug-related	All	Study drug-related
Any adverse events	29 (56.9)	4 (7.8)	25 (49.0)	11 (21.6)
Discontinued because of adverse event	9 (17.6)	1 (2.0)	1 (2.0)	0
Serious adverse event	0	0	0	0
Adverse events ≥3% in any groups				
Nasopharyngitis	4 (7.8)	0	5 (9.8)	0
Headache	1 (2.0)	1 (2.0)	2 (3.9)	1 (2.0)
Upper respiratory tract inflammation	3 (5.9)	0	2 (3.9)	0
Ulcerative colitis	9 (17.6)	0	2 (3.9)	0
Nausea	1 (2.0)	0	2 (3.9)	2 (3.9)
Abdominal bloating	2 (3.9)	1 (2.0)	1 (2.0)	1 (2.0)
Upper abdominal pain	2 (3.9)	0	0	0
Blood amylase increased	0	0	2 (3.9)	2 (3.9)
Blood lactate dehydrogenase increased	0	0	2 (3.9)	2 (3.9)
C-reactive protein increased	1 (2.0)	0	2 (3.9)	1 (2.0)
White blood cell count increased	0	0	2 (3.9)	1 (2.0)

specifically related to biologics, such as immunogenicity, infusion reaction, and loss of efficacy related to the production of antidrug antibodies, could be observed in the clinical practice.[12] Antibodies to infliximab develop in 8%–60% of patients with IBD.[12] The increase in the number of patients with loss of response or intolerance might also be observed during treatment with the anti-integrin antibodies in the future.[9,24] Orally active small-molecule medicine with pharmacologic activity similar to biologics could be beneficial for patients with IBD by overcoming these problems of biologics.

As for safety of AJM300, there was no serious adverse event in this study. The incidence of adverse events in the active treatment group was similar to that in the placebo group. Only 1 patient (2.0%) receiving the active treatment discontinued the study due to an adverse event, and 9 patients (17.6%) receiving placebo discontinued the study, mainly due to aggravation of UC. All drug-related adverse events were mild and self-limiting. Neither infection nor neurologic symptom was observed.

The most concerning adverse event of α4 integrin blockade is the development of PML, which is a demyelinating disorder of the central nervous system caused by JCV infection and reactivation. PML occurs in severely immunocompromised patients, such as those with acquired immune deficiency syndrome, and with use of immunosuppressants or some biologics, and usually leads to death or severe disability. As of February 2012, two hundred and twelve confirmed PML cases among 99,751 patients treated with natalizumab were reported (2.1 cases per 1000 patients).[25] Although no cases of PML have yet been observed among approximately 500 IBD patients and healthy volunteers treated with AJM300 for a maximum of 8 weeks in the clinical studies, such short-term safety data do not preclude the possibility of PML risk with this agent. Clinical development of AJM300 should be carefully conducted under a risk-management program to mitigate the potential risk for PML.

Based on the large post-marketing surveillance data derived from >100,000 natalizumab-treated patients, 2 especially important findings have been obtained about risk factors for PML. First, no cases of PML have been observed with the therapy for ≤8 months.[26] Second, the risk of PML was much lower among the patients who were negative for anti-JCV antibodies than those who were positive (the incidence of PML; 0/1000; 95% CI: 0–0.32 and 3.87/1,000; 95% CI: 2.91–5.05, respectively).[25,26] A prospective study further confirmed that no cases of PML were observed in natalizumab-treated patients who were negative for anti-JCV antibodies.[27] These data suggest that the potential PML risk of AJM300 may be minimized by limiting treatment duration to <8 months. In addition, the measurement of the anti-JCV antibodies might provide information helpful for deciding whether to continue treatment with this agent. Currently, the clinical development of AJM300 should be focused on induction therapy in active UC patients, with PML risk stratification by measurement of anti-JCV antibodies. A long-term safety study should be conducted to preclude a possible increased risk of PML with this agent. The potential risk of PML should be mitigated by a special restricted distribution program under a risk-management action plan in clinical practice as well as in clinical trials. The patients who were in immunocompromised conditions or treated with concomitant immunosuppressants or anti-TNFα antibodies should be precluded. Furthermore, the patients with any neurologic symptom should be precluded to enable timely detection of new neurologic symptoms that suggest PML development. In the event of suspected PML, early removal of drug from the body might lead to more acceptable outcomes of the complication.[26,28] For instance, a timely discontinuation of treatment with natalizumab followed by plasma exchange and immunoadsorption has been recommended.[26,28] AJM300 may have an advantage in the outcome of PML, because of extremely shorter duration of pharmacodynamic action (within a day after the last dose in this study) than natalizumab (approximately 4 months).[29] However, careful monitoring and risk minimization strategies for PML will still be required to use this drug.

We concluded that the dosage of 960 mg 3 times daily was optimal, because this dose was well tolerated and demonstrated the expected clinical efficacy. Furthermore, the increase in peripheral lymphocyte counts, which is one of the pharmacologic activities of α4 integrin blockade, was maintained throughout the day at this dose regimen.

This study has the following limitations: small sample size (a total of 102 patients, 51 patients per group), a short period of study drug medication (8 weeks), and extremely low enrollment of an inadequate response or intolerance to corticosteroids. There was no information on medical history regarding an inadequate response or intolerance to corticosteroids, immunosuppressants, and anti-TNFα antibodies. Larger or longer-term clinical trials are required to confirm the efficacy and safety of AJM300.

Most of the patients enrolled in this study had an inadequate response to mesalamine. In the treatment of UC, corticosteroids have been, for decades, the first and almost exclusive therapeutic option for such patients. Given the diverse adverse effects of corticosteroids, reasonable alternatives to them have been desired. AJM300 can be administered on an outpatient basis without any infusion equipment or instruction. Thus, AJM300 could be suitable for induction therapy in patients with moderately active UC refractory to mesalamine.

In conclusion, oral treatment with the small-molecule α4 integrin antagonist AJM300 was safe, well tolerated, and more efficacious than placebo for inducing clinical response, clinical remission, and mucosal healing in patients with moderately active UC in this study. AJM300 may become a novel oral therapeutic option especially for patients who had an inadequate response to mesalamine with moderately active UC, and phase 3 study for induction therapy is currently ongoing in Japan.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.08.044.

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Conflicts of interest

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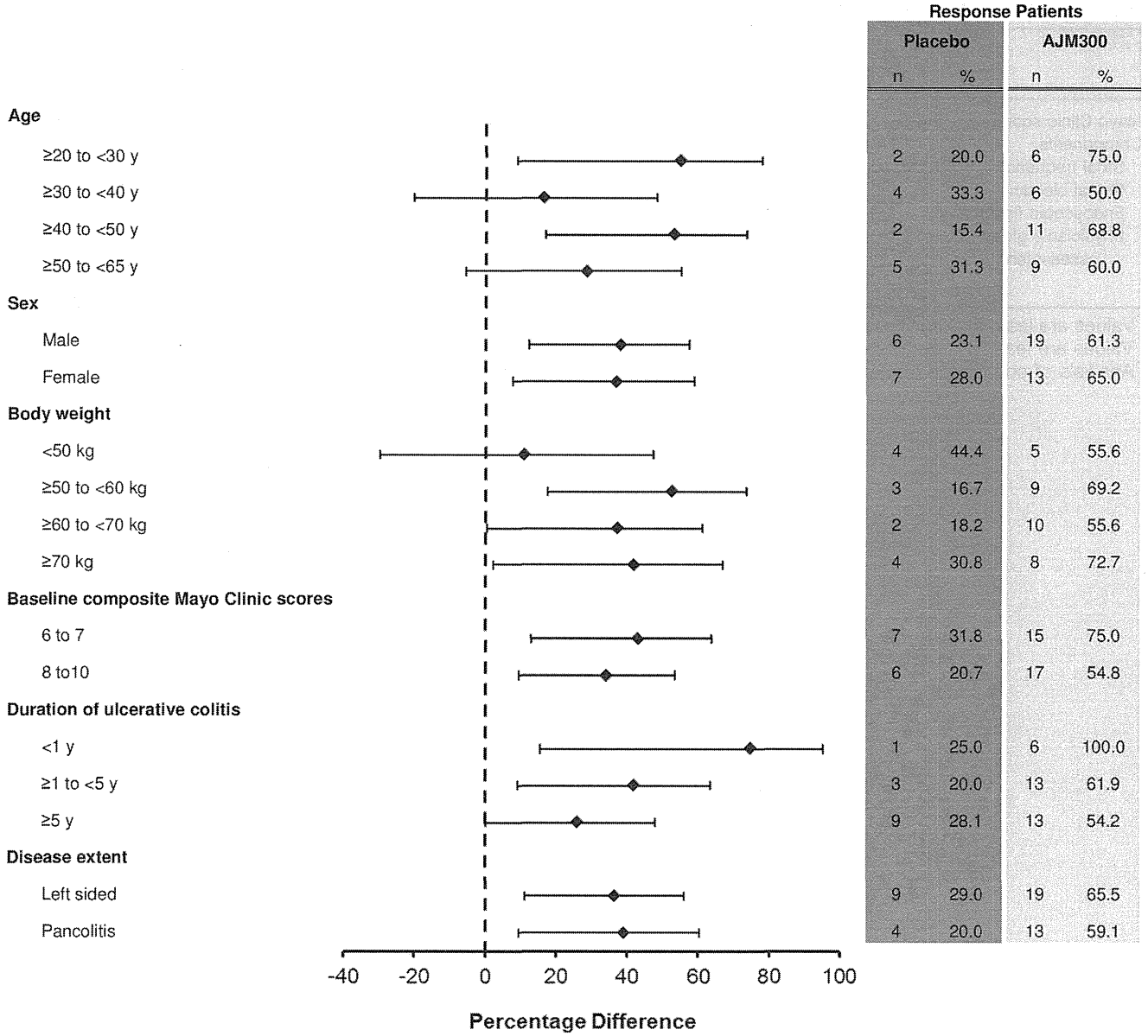

Supplementary Figure 1. Subgroup analysis. Plot of risk differences (*dots*) and 95% CIs (horizontal bars) for comparing the proportion of patients with clinical response between AJM300 and placebo at week 8 by baseline characteristics.

Supplementary Table 1. Change from Baseline to Week 8 in the Mayo Clinic Score and Each Subscore

	Placebo[a]	AJM300[a]	Difference from placebo[b]	95% CI	*P* value[c]
Mayo Clinic score	−1.87 ± 0.61 (44)	−4.05 ± 0.60 (47)	−2.18 ± 0.49	−3.14 to −1.21	<.0001
Components					
Stool frequency	−0.36 ± 0.22 (51)	−1.06 ± 0.22 (51)	−0.70 ± 0.17	−1.03 to −0.36	<.0001
Rectal bleeding	−0.38 ± 0.23 (51)	−0.96 ± 0.23 (51)	−0.58 ± 0.18	−0.93 to −0.22	.0016
Endoscopic findings	−0.55 ± 0.18 (44)	−1.01 ± 0.18 (47)	−0.46 ± 0.15	−0.75 to −0.17	.0024
Physician's global assessment	−0.53 ± 0.16 (51)	−0.96 ± 0.16 (51)	−0.43 ± 0.13	−0.67 to −0.18	.0009

[a]Values are least squares mean ± standard error (n).
[b]Values are least squares mean ± standard error.
[c]Analysis of covariance models were adjusted for stratification factors at randomization.

Supplementary Table 2. Change From Baseline to Week 8 in Riley Score and Each Subscore

	Placebo[a]	AJM300[a]	Difference from placebo[b]	95% CI	*P* value[c]
Riley score	−0.94 ± 1.03 (40)	−3.03 ± 1.01 (46)	−2.09 ± 0.86	−3.79 to −0.39	.0017
Histological features					
Round cells in the lamina propria	−0.47 ± 0.22 (42)	−0.93 ± 0.21 (46)	−0.46 ± 0.18	−0.81 to −0.10	.012
Polymorphonuclear cells in the lamina propria	−0.33 ± 0.26 (42)	−0.89 ± 0.25 (46)	−0.56 ± 0.21	−0.97 to −0.14	.0096
Mucin depletion	−0.085 ± 0.251 (42)	−0.50 ± 0.25 (46)	−0.41 ± 0.21	−0.82 to −0.0052	.047
Crypt abscesses	−0.31 ± 0.26 (42)	−0.65 ± 0.25 (46)	−0.34 ± 0.21	−0.77 to 0.086	.12
Surface epithelial integrity	−0.38 ± 0.30 (42)	−0.16 ± 0.29 (46)	−0.22 ± 0.24	−0.70 to 0.26	.36
Crypt architectural irregularities	−0.17 ± 0.19 (42)	−0.23 ± 0.19 (46)	−0.053 ± 0.160	−0.37 to 0.27	.74

NOTE. Subjects who did not have biopsy and had inadequate biopsy at week 0 or 8 were excluded from this analysis.
[a]Values are least squares mean ± standard error (n).
[b]Values are least squares mean ± standard error.
[c]Analysis of covariance models were adjusted for stratification factors at randomization.

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ORIGINAL ARTICLE

NUDT15 R139C causes thiopurine-induced early severe hair loss and leukopenia in Japanese patients with IBD

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The efficacy of thiopurines, including azathioprine (AZA) and 6-mercaptopurine (6MP), has been demonstrated for the treatment of inflammatory bowel disease (IBD). The most common and serious adverse event of treatment with thiopurines altered by doctors is leukopenia. Hair loss is also a serious event that could be a critical reason for patients to decline thiopurine treatment. Thiopurine-induced severe hair loss causes cosmetic problems, and it takes a long time to recover. In a recent study, NUDT15 R139C was strongly associated with thiopurine-induced leukopenia in Korean and Caucasian populations. In this study, we performed an association study to investigate and replicate the association of R139C with adverse events of thiopurines in Japanese patients. A total of 142 Japanese patients with IBD, with histories of thiopurine treatment, were examined. NUDT15 R139C was genotyped using a custom TaqMan genotyping assay. Adverse events including leukopenia were reviewed from medical records. The 6MP dose was adjusted to AZA equivalents by multiplying with 2 as a thiopurine dose. Five patients developed severe hair loss and all of them were risk homozygous (T/T) for R139C. No early severe hair loss was observed in patients with the C/T or C/C genotype ($P=3.82\times10^{-16}$, odds ratio = 212). The association of R139C with early (< 8 weeks) leukopenia (white blood cells < 3000 mm^{-3}), which was previously reported in Korean patients, was replicated in our Japanese IBD cohort ($P=1.92\times10^{-16}$, odds ratio = 28.4). However, we could not confirm the association with late leukopenia in the Japanese subjects. Patients with the C/T genotype discontinued treatment or required thiopurine dose reduction significantly earlier than patients with the C/C genotype ($P=1.45\times10^{-4}$); however, on manipulating the doses, there was no significant difference in the thiopurine continuation rates between the groups. In the maintenance period, the frequencies of 6MP usage were higher, and the doses of thiopurines were significantly lower in patients with the C/T genotype than in those with the C/C genotype (0.574 ± 0.316 mg kg^{-1} per day vs 1.03 ± 0.425 mg kg^{-1} per day, $P=6.21\times10^{-4}$). NUDT R139C was significantly associated with early severe hair loss in Japanese patients with IBD. We also verified the previously reported association of R139C with early leukopenia in a different East Asian population. It is recommended that treatment with thiopurines should be avoided for patients with the T/T genotype. Low-dose 6MP (0.2–0.3 mg kg^{-1} per day) could be used rather than AZA for the patients with C/T genotype to continue thiopurine treatments. However, late leukopenia and other several adverse events could not be completely predicted by R139C genotypes.

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INTRODUCTION

Thiopurines, including azathioprine (AZA) and 6-mercaptopurine (6MP), have been used for both induction and maintenance therapy in patients with inflammatory bowel disease (IBD),[1] including Crohn's disease (CD)[2,3] and ulcerative colitis.[4] Infliximab has recently been also used to treat IBD, and the Study of Immunomodulator Naive Patients in Crohn's Disease trial demonstrated that Infliximab plus AZA was superior in the treatment of CD to Infliximab monotherapy, which was in turn superior to AZA monotherapy for the short-term induction of remission.[5] However, it is well known that treatment with thiopurines must be discontinued in some patients because of adverse events such as leukopenia, gastrointestinal intolerance, pancreatitis and liver dysfunction.[6,7] The most common and serious adverse event of treatment with thiopurines altered by doctors is leukopenia. Hair loss is also a serious event that could be a critical reason for patients to decline thiopurine treatment. Thiopurine-induced severe hair loss causes cosmetic problems, and it takes a long time to recover. To avoid these adverse events, several genetic studies have been performed.[8–11] In Caucasian patients, the genetic polymorphisms of thiopurine S-methyltransferase, an enzyme involved in the inactivation of 6MP, were reported to be associated with thiopurine-induced leukopenia;[11] however, the association was not replicated in Japanese subjects.[12,13] In a recent study, NUDT15 R139C was strongly associated with thiopurine-induced leukopenia in Koreans.[14] It is well known that most genes conferring susceptibility to IBD were common in Japanese and Korean individuals. However, the association of ATG16L1-T300A with CD in Koreans was recently reported,[15] although the association has not been confirmed in Japanese patients.[16,17] This finding implicated that the genetic background of Japanese individuals is extremely similar, but not identical, to that of Koreans. The findings of genetic susceptibilities in Korean individuals need to be confirmed in Japanese populations, even though these two populations are both East Asians. In this report, we confirmed the association of R139C with thiopurine-induced

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leukopenia and determined whether it is associated with hair loss in Japanese patients.

METHODS

Subjects

Blood samples were obtained from 142 patients with IBD (CD, 116; ulcerative colitis, 25; and IBD unclassified, 1) who had histories of thiopurine treatment and who visited Tohoku University Hospital between April 2002 and September 2014. On the basis of the previous report about leukopenia in the Korean population, we performed a power analysis and confirmed that our cohort would provide almost 100% power in the analyses. The self-reported ethnicity of all patients was Japanese. The diagnosis of CD and ulcerative colitis was made on the basis of clinical symptoms and endoscopic, radiographic and histological findings according to conventional criteria proposed by the Japanese Ministry of Health, Labour and Welfare. Genomic DNA was obtained from peripheral blood leukocytes by standard phenol–chloroform extraction precipitation or by utilizing an NA1000 Automated Nucleic Acid Extraction Machine (Kurabo, Osaka, Japan) or PAXgene DNA Kit (BD, Franklin Lakes, NJ, USA). The study was approved by the Ethics Committee of Tohoku University School of Medicine, and all patients gave written informed consent to participate in this study.

Thiopurine treatments and adverse events

The initial and maintenance treatment doses, duration of treatment, combined usage of anti-tumor necrosis factor-α drugs (Infliximab or Adalimumab) and reason for treatment discontinuation were reviewed from medical records. Maintenance dose of thiopurines was defined as the final dose given to the patients who could continue thiopurines for more than 1 year. The 6MP dose was adjusted to AZA equivalents by multiplying with 2. Leukopenia and severe hair loss as serious adverse events of thiopurine treatment were also reviewed from medical records. Leukopenia was graded by common toxicity criteria[18] as follows: grade 2, 2000–3000 mm^{-3}; grade 3, 1000–2000 mm^{-3}; and grade 4, <1000 mm^{-3}. Severe hair loss was defined as objective hair loss such that patients (especially women) may need to wear wigs, and needed a few months to recover. Self-reported subjective hair loss was excluded because it was hard to evaluate, and did not cause cosmetic problems.

Brief protocol of thiopurine usage

Thiopurine treatments were used for the patients following the treatment guidelines of CD or Ulcerative Colitis in Japan. We usually start thiopurines at a dose of 0.5–1.0 mg kg^{-1} per day (as AZA dose) and check adverse events at 7–14 days from the initiation. If patients have no adverse events, we increase thiopurines at a rate of 0.5 mg kg^{-1} per day every 4 weeks or a few months up to 2.0–3.0 mg kg^{-1} per day. We monitor complete blood counts, serum levels of amylase and liver enzymes every month. If patients have severe leukopenia (Grade 3 or 4), severe hair loss, acute pancreatitis or anxiety regarding the continuation of thiopurines, we discontinue their treatment. If the adverse events are not so severe (that is, Grade 2 Leukopenia, slight elevation of serum amylase level), we decrease thiopurines at a rate of 0.5 mg kg^{-1} per day.

Genotyping

Genotyping of NUDT15 R139C (rs116855232) was examined using a Custom TaqMan SNP Genotyping Assay kit and a Step-One Plus Real Time PCR system (Life Technologies, Carlsbad, CA, USA). The sequences of the forward and reverse primers and two allele-specific oligonucleotide probes labeled with a fluorescent reporter dye (FAM or VIC) specific for rs116855232 were 5′-CCCCTGGACCAGCTTTTCTG-3′, 5′-CCACCAGATGG TTCAGATCTTCTTTAAA-3′, 5′-AAACAACGCAGTCCC-3′ (VIC), and 5′- TTTAAA CAACACAGTCCC-3′ (FAM), respectively. We also determined TPMT genotypes by rs1142345, using a TaqMan Drug Metabolism Genotyping Assays (C_19567_20, Life Technologies). The thermal cycler condition for polymerase chain reaction was 40 cycles of 15 s at 95 °C for denaturation and 60 s at 60 °C for annealing and extension.

Statistical analysis

The baseline characteristics of the patients initially treated with AZA or 6MP were compared using the chi-squared or Student's *t*-test. Patient age at the initiation of thiopurine treatment, patient gender, the initial dose of thiopurines and the frequencies of leukopenia and severe hair loss for each genotype of R139C were compared by analysis of variance or 3×2 chi-squared tests in univariate analyses. Logistic regression analyses were also performed to identify the associations of leukopenia with each genotype and other factors in multivariate analyses. In addition, 2×2 chi-squared tests were performed to identify the association of the risk allele with adverse events. Kaplan–Meier and log-rank test were used for cumulative thiopurine continuation rate analyses. *P*-values <0.05 were considered significant. All analyses were performed using R software version 2.14.1.

RESULTS

Seven patients were excluded because of incomplete leukocyte data. The baseline characteristics of the patients are summarized in Table 1. In total, 112 of 135 (83.0%) patients were initially treated with AZA, and the remaining patients were first treated with 6MP. The percentage of females who received 6MP was significantly higher than that of AZA-treated females. No significant differences were observed in the ages and initial doses between these two groups. Mean and median observation time of the all patients were 1172 and 848 days.

Severe hair loss and early leukopenia was significantly associated with NUDT15 R139C

Information about thiopurine usage and the adverse events of patients with each R139C genotype is summarized in Table 2. The age of patients at the initiation of thiopurine treatment, the proportions of patients who first received AZA or 6MP, gender and the initial treatment dose were not significantly different among the genotypes. Severe hair loss was observed in five patients, all of whom were mutant homozygous (T/T) for R139C ($P=4.84\times10^{-30}$). Thirty-four patients (25.2%) developed leukopenia (grade 2 or higher) in our cohort, and R139C was significantly associated with leukopenia in Japanese subjects as observed in Koreans ($P=5.49\times10^{-5}$).

TPMT variant was confirmed not to be associated with leukopenia

Most patients were homozygous for wild allele (TPMT *1/*1), and only two patients showed heterozygous (TPMT *1/*3C). Both of these two patients discontinued thiopurines within 8 weeks (17 and 29 days, respectively), but the reasons for discontinuation were not hair loss or leukopenia (inefficacy and nausea). Thus, there was no significant association of TPMT*3C with thiopurine-induced leukopenia, as previously reported.[12,13]

Table 1. Patient characteristics

	AZA	*6MP*	*Total*	P-*values*
Total (*n*)	112	23	135	
Gender (M/F)	86/26	12/11	98/37	0.0313
Age[a]	34.7 ± 11.5	38.2 ± 14.9	35.3 ± 12.2	0.218
Initial dose[b] (mg kg^{-1} per day)	0.840 ± 0.281	0.779 ± 0.121	0.829 ± 0.261	0.317
Diagnosis				
CD	90	21	111	
UC	21	2	23	
IBDU	1	0	1	

Abbreviations: AZA, azathioprine; CD, Crohn's disease; F, female; IBD, inflammatory bowel disease; M, male; 6MP, 6-mercaptopurine; UC, ulcerative colitis. [a]Mean ± s.d. [b]The 6MP dose was adjusted to AZA equivalents by multiplying with 2.

Table 2. Comparison of thiopurine usage and adverse events according to R139C genotypes

Genotype of R139C	*C/C*	*C/T*	*T/T*	*P-values*
Number of the patients (genotype frequencies)	107 (79.3%)	23 (17.0%)	5 (3.7%)	
Age at starting thiopurines[a]	34.6 ± 12.0	38.6 ± 14.2	35.4 ± 6.80	0.373
Gender (M/F)	74/33	20/3	4/1	0.206
Combination therapy with anti-TNF drugs[b] (%)	39 (36.4%)	6 (26.1%)	2 (40.0%)	0.620
AZA/6MP	89/18	18/5	5/0	0.499
Initial dose of AZA (mg kg^{-1} per day)[a]	0.818 ± 0.237	0.920 ± 0.332	0.927 ± 0.661	0.298
Initial dose of 6MP(mg kg^{-1} per day)[a]	0.385 ± 0.0559	0.407 ± 0.0787	–	0.473
Initial dose of AZA (including 6MP)[a,c]	0.810 ± 0.221	0.897 ± 0.303	0.927 ± 0.661	0.249
Early severe hair loss	0 (0%)	0 (0%)	5 (100%)	4.84×10^{-30}
Leukopenia (%)	19 (17.8%)	10 (39.1%)	5 (100%)	1.61×10^{-5}
Grade 4 (WBC < 1000)	0	0	3	
Grade 3 (WBC < 2000)	1	0	2	
Grade 2 (WBC < 3000)	18	10	0	

Abbreviations: 6MP, 6-mercaptopurine; AZA, azathioprine; F, female; M, male; TNF, tumor necrosis factor; WBC, white blood cell. [a]Mean ± s.d. [b]Anti-TNF drugs (Infliximab or Adalimumab) usage during initiation period of thiopurines. [c]The 6MP dose was adjusted to AZA equivalents by multiplying with 2.

Table 3. Association of early and late leukopenia with R139C genotypes

					Allelic associations		
NUDT15 R139C genotype	*C/C*	*C/T*	*T/T*	*P-values*	*Allele T frequencies*	*P-values*	*Odds ratios (95% CI)*
Early leukopenia (< 8 weeks, G2 or above)	1 (0.93%)	4 (17.4%)	5 (100%)	1.92×10^{-16}	70.0%	4.38×10^{-15}	28.4 (9.78–82.3)
Early G4 (WBC < 1000)	0	0	3				
Early G3 (WBC < 2000)	0	0	2				
Early G2 (WBC < 3000)	1	4	0				
No early leukopenia	106	19	0		7.60%		
Late leukopenia (⩾8 weeks, G2 or above)	18 (19.4%)	6 (33.3%)	–	0.314	12.5%	0.337	–
Late G4 (WBC < 1000)	0	0	–				
Late G3 (WBC < 2000)	1	0	–				
Late G2 (WBC < 3000)	17	6	–				
No late leukopenia	75	12	–		6.90%		
Early severe leukopenia (G3 or G4)	0 (0%)	0 (0%)	5 (100%)	4.84×10^{-30}	100%	3.82×10^{-16}	212 (12.1–3737)

Abbreviations: CI, confidence interval; WBC, white blood cell.

R139C was associated with early but not late leukopenia

R139C genotypes were significantly associated with early leukopenia (leukopenia developing within 8 weeks) (Table 3), and all of the patients with the T/T genotype developed early severe (Grade 3 or 4) leukopenia ($P = 3.82 \times 10^{-16}$, odds ratio = 212). These associations were not observed for late leukopenia ($P = 0.907$). In the logistic regression analysis with gender, age, the initial dose of thiopurines, combined usage of anti-tumor necrosis factor-α drugs and R139C risk allele counts, only R139C was significantly associated with early leukopenia (Table 4). Comparing C/C and C/T genotypes, patients with the C/T genotype developed early leukopenia more frequently than those with the C/C genotype ($P = 6.71 \times 10^{-3}$, odds ratio = 22.3).

Table 4. Multivariate association analyses of early leukopenia with R139C genotypes

Factor	*P-values*	*Odds ratio (95%CI)*
Gender (Male)	0.463	–
Age at starting thiopurines	0.512	–
AZA dose[a]	0.347	–
Anti-TNFα therapy	0.698	
R139C risk allele counts	4.65×10^{-4}	51.9 (9.46–1256)

Abbreviations: AZA, azathioprine; CI, confidence interval; TNF, tumor necrosis factor. [a]The 6MP dose was adjusted to AZA equivalents by multiplying with 2.

AZA continuation rates among patients heterozygous for R139C were significantly low because of leukopenia

Comparing cumulative continuation rates for the initial AZA dose, patients who were heterozygous (C/T) for R139C required treatment discontinuation or dose modification earlier than patients who were wild homozygous for R139C (C/C) (Figure 1a, $P = 1.45 \times 10^{-4}$). The most frequent reason for AZA discontinuation was leukopenia for patients with C/T and C/C genotypes (Figures 2a and b), and treatment discontinuation was more frequent for C/T genotype, albeit without significance (46 vs 23%, $P = 0.15$).

Thiopurine treatment could be continued by adjusting the dose in patients heterozygous for R139C

When the period after adjusting thiopurine doses and/or switching to 6MP was included, the cumulative thiopurine continuation rates in the patients who were heterozygous for R139C improved (Figure 1b), especially after 8 weeks (Figure 1c). In

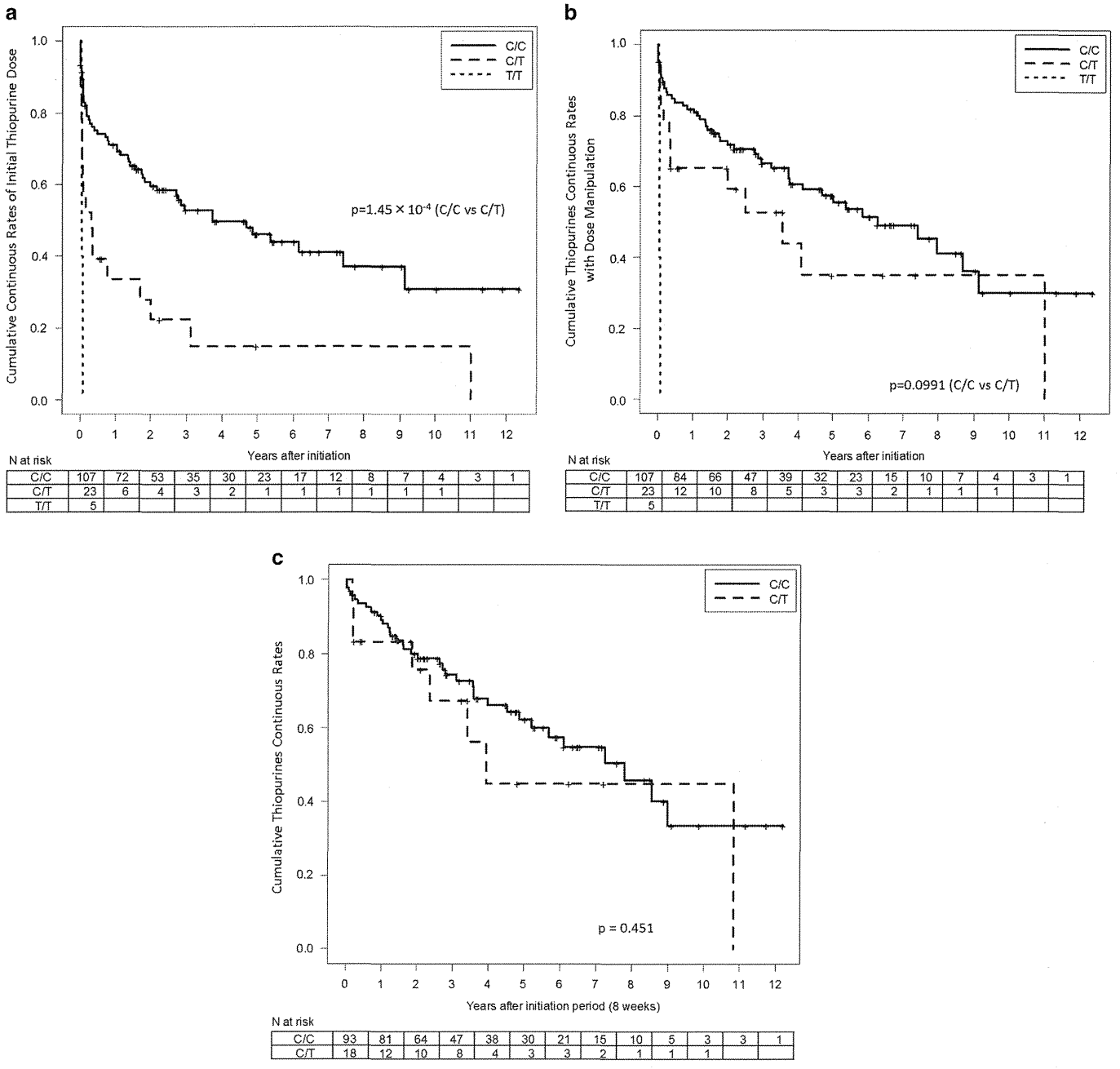

Figure 1. Cumulative continuation rates of thiopurines according to R139C genotypes. (**a**) Cumulative continuation rates at the initial thiopurine doses. (**b**) Cumulative continuation rates after dose manipulation, including switching from azathioprine and 6-mercaptopurine. (**c**) Continuation rates 8 weeks after the initiation of thiopurine treatment. In total, 24 of 135 patients were excluded owing to the failure of thiopurine treatment within 8 weeks.

the maintenance period, the frequencies of 6MP usage were higher and the doses of thiopurines were significantly lower in patients with the C/T genotype than in those with the C/C genotype (Table 5). Forty-five percent of patients with the C/T genotype, who could not continue thiopurine treatment for more than 1 year, discontinued thiopurines within 8 weeks after the initiation. Initial doses of the patients with the C/T genotype, who discontinued thiopurines within 8 weeks, were significantly higher than that of patients who could continue thiopurines for more than 8 weeks (1.16 mg kg^{-1} per day and 0.804 mg kg^{-1} per day, respectively, $P = 0.0245$).

DISCUSSION

The efficacy of thiopurines in the treatment for IBD is endorsed by several clinical studies, but the adverse events cannot be ignored. Even after the induction of thiopurine treatment, some patients discontinued treatment for reasons of inconvenience. Excluding leukopenia, the most frequent reason for thiopurine discontinuation was 'Personal Circumstances,' including anxiety for adverse events such as hair loss. Therefore, to reduce anxiety concerning adverse events, companion diagnostic tests have been expected. In this study, we found a novel, significantly strong association of

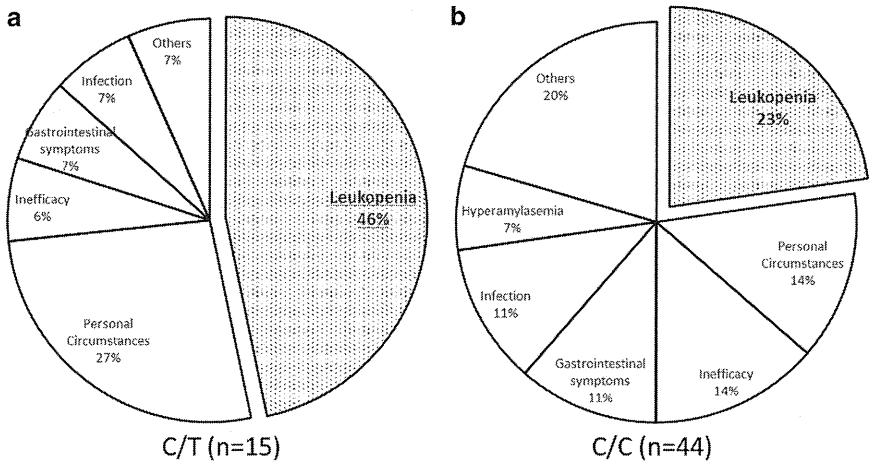

Figure 2. Reasons for the discontinuation of thiopurine treatment. Personal circumstances included anxiety for adverse events, a desire to bear children and non-compliance with treatment.

Table 5. Maintenance doses of thiopurines

Genotype	*C/C*	*C/T*	P
n	84	12	
AZA/6MP	65/19	6/6	0.0949
AZA dose[a] (mg kg^{-1} per day)	1.03 ± 0.425	0.574 ± 0.316	6.21×10^{-4}

Abbreviations: AZA, azathioprine; 6MP, 6-mercaptopurine. [a]Mean ± s.d. The 6MP dose was adjusted to AZA equivalents by multiplying with 2.

NUDT15 R139C with thiopurine-induced early severe hair loss. We also replicated the association of R139C with thiopurine-induced early leukopenia in Japanese subjects; it is an independent and first verification of association with leukopenia in a different East Asian population. All of the patients, who were mutant homozygous (T/T) for R139C, developed both severe leukopenia and severe hair loss. No patients with heterozygous (C/T) or wild-type homozygous (C/C) genotypes developed early 'severe' leukopenia or hair loss.

Comparing the patients with C/T and C/C genotypes, the patients with the C/T genotype developed grade 2 leukopenia frequently within 8 weeks, and the rate treatment continuation at the initial dose of thiopurines was significantly lower among these patients than among those with the C/C genotype. Some of these patients discontinued thiopurine therapy, but the others could continue thiopurine treatment upon dose reduction. Manipulating the doses of thiopurines could shrink the difference in continuation rates between patients with the C/T and C/C genotypes, and significant difference in continuation rates was noted. Hibi *et al.*[19] reported that even low-dose AZA was effective as a maintenance therapy in patients with ulcerative colitis. Therefore, patients with the C/T genotype could be treated with thiopurines using low-dose thiopurines. However, the clinical efficacy of low-dose thiopurines for patients with IBD and the C/T genotype is not clear, especially for patients with CD. The sample size of our cohort is not sufficient to investigate the clinical therapeutic effects of thiopurines according to R139C genotypes, and therefore, further analyses, such as multicenter analyses, should be performed.

Late leukopenia was also associated with R139C in the previous report on Koreans,[14] but the association was not as strong as that for early leukopenia. In this study, we could not replicate the association of R139C with late leukopenia. More than half of the cases of late leukopenia (14 of 24 cases) developed later than 1 year after the initiation of thiopurine treatment, and the mean time to develop late leukopenia was 97.9 weeks. Only one patient developed grade 3 leukopenia after 8 weeks, but the patient was wild-type (non-risk) homozygous for R139C. From these findings, late severe leukopenia could be developed in patients even when they were non-risk homozygous for R139C, and the pathogenesis of late leukopenia could be different from that of early leukopenia.

It is important to investigate the toxicity of thiopurines as a result of allergic reaction or over-dosing. We were unfortunately unable to check 6-thioguanine nucleotides levels of the patients. However, considering the function of NUDT15, it may not be associated with the metabolic pathway of thiopurines. NUDT15 is considered to degrade 8-oxo-dGTP and 8-oxo-dGDP which induce base mispairing,[20] so NUDT15 may prevent genetic information from the untoward effects of endogenous oxygen radicals. In a previous report, it was demonstrated that NUDT15 with R139C mutation may have a dominant role in thiopurine-induced cytotoxicity.[14] Therefore, we speculate that early severe hair loss and leukopenia did not result from allergic reaction or overdosing of 6-thioguanine nucleotides and may have been caused because of the intolerance in patients.

Severe hair loss and leukopenia were two of the typical adverse events in thiopurine treatment, but the associations of other adverse events with genetic polymorphisms have also been investigated. For example, MTHFR C677T and A1298C were reported to be associated with liver dysfunction during maintenance therapy for acute lymphoblastic leukemia in Japanese,[21] and rs2413739 in PACSIN2 was reported to be associated with gastrointestinal toxicity.[22] However, these associations were not as strong as NUDT15 with hair loss and leukopenia, and the results of PACSIN2 were controversial.[23] Pancreatitis is another critical adverse event of thiopurine treatment. Ahmad *et al.*[8] recently reported that the HLA-DQA1*02:01–HLA-DRB1*07:01 haplotype was significantly associated with thiopurine-induced pancreatitis. Only three patients had pancreatitis in our study, and all of them were wild-homozygous for NUDT15 R139C. To determine whether these patients carry the risk haplotype of HLA, we performed low-resolution HLA-DRB1 genotyping by PCR amplification with the sequence-specific primer reported by Bunce *et al.*[24] Their genotypes were *08/*09, *04/*13 and *14/*15, respectively, but none of them carried the DRB1*07 allele. However, our cohort is limited and too small to confirm this association in the Japanese population. Genome-wide scan with a larger cohort is expected to be performed for clarifying the genetic susceptibility to

thiopurine-induced pancreatitis in the Japanese population. These genetic associations of thiopurine toxicity may only explain a part of the adverse events and may be multifactorial. By contrast, early severe hair loss and leukopenia in the Japanese was almost completely predictable by NUDT15 R139C, which means that these may be monogenic.

It is still unknown how NUDT15 R139C causes severe hair loss and leukopenia. Considering some functional analyses of previous reports, we can speculate that thiopurines may cause inappropriate and excessive apoptosis of cells that divide rapidly, such as hair matrix cells and leukocytes, through the inhibition of DNA synthesis and DNA repairing. However, the exact function of NUDT15 in human subjects is still unclear, and further functional analyses of NUDT15 and its polymorphism are required.

From the results in this study, the genotyping of NUDT15 R139C could be a reliable and potent companion diagnostic test for thiopurine-induced early severe hair loss and leukopenia in Japanese subjects. It is recommended that treatment with thiopurines should be avoided for patients with the T/T genotype and low-dose 6MP (0.2–0.3 mg kg^{-1} per day) should be used rather than AZA for the patients with the C/T genotype. However, late leukopenia could not be completely predicted by R139C genotypes; thus, we must still pay attention to late leukopenia even when patients' white blood cell counts remain stable. It is hoped that efforts will be made to find new diagnostic markers for late leukopenia.

CONCLUSION

NUDT R139C T/T genotype showed complete association with early severe hair loss in Japanese patients with IBD. In addition, the association of R139C with thiopurine-induced leukopenia was confirmed in Japanese patients with IBD. R139C genotyping can be a companion diagnostic marker for both thiopurine-induced severe hair loss and leukopenia in Japanese patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE—ALIMENTARY TRACT

First trough level of infliximab at week 2 predicts future outcomes of induction therapy in ulcerative colitis—results from a multicenter prospective randomized controlled trial and its post hoc analysis

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Abstract

Background Infliximab (IFX) is one of the treatments of choice for corticosteroid-refractory and corticosteroid-dependent ulcerative colitis (UC). A high serum trough level of IFX (TL) is reported to be associated with sustained efficacy during maintenance treatment. As part of a phase 3 randomized controlled trial of IFX in UC, we assessed the predictive value of the first TL at week 2 for short- and long-term response.

Methods Patients received intravenous IFX 5 mg/kg or placebo at weeks 0, 2, and 6. Patients with evidence of a response by week 8 continued treatment at weeks 14 and 22. TL was measured by enzyme-linked immunosorbent assay. Post hoc analysis was then performed for TL and clinical outcomes.

Results Clinical response rate at week 8, the primary end point, was significantly higher in the IFX group than placebo ($p = 0.005$). The incidence of adverse events between groups was similar. Week 2 TL was significantly associated with a 14-week clinical activity index (CAI) remission. In multiple logistic regression analysis, the week 2 TL-to-CAI ratio (TL/CAI, odds ratio 8.07; 95 % confidence interval 2.84–27.07, $p < 0.001$) was an independent factor correlating with 14-week CAI remission. The week 2 TL and TL/CAI were also significantly associated with 30-week mucosal healing.

Conclusions IFX was confirmed to be effective and safe in this population. Our results suggest that the first TL at week 2, in combination with clinical evaluation, is useful for predicting both short- and long-term outcomes, allowing an earlier decision between continuing IFX or switching to other options.

Keywords Ulcerative colitis · Infliximab · Therapeutic drug monitoring · Primary response · Mucosal healing

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are two major forms of chronic inflammatory bowel disease [1]. Although their etiology remains unknown, immunological, genetic, and environmental factors are considered to play important roles in their pathogenesis [2, 3]. Tumor necrosis factor-alpha (TNF-α) is one of the cytokines reported to be increased in both UC and CD [4, 5], and TNF-α blockade has emerged as an effective therapeutic strategy for both diseases. The first use of the anti-TNF-α antibody

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infliximab (IFX) for human inflammatory bowel disease was reported in a pediatric patient with severe CD in 1997 [6]. Efficacy in CD was confirmed in the subsequent ACCENT I and II randomized controlled trials [7]. Efficacy for UC was studied in the 54-week ACT 1 and 30-week ACT 2 trials, in which the IFX groups showed statistically significant increases in the proportion of patients with a clinical response, clinical remission, and mucosal healing (MH) compared with placebo [8].

Intravenous corticosteroid administration remains the first-line therapy in UC patients with acute moderate-to-severe disease and is reported to be effective in more than half of them [9]. However, a certain proportion of patients is refractory to corticosteroid treatment. Moreover, even among initial responders, approximately one-third of patients cannot withdraw from corticosteroids without a symptom flare, or experience symptoms after steroid discontinuation, and are therefore considered steroid-dependent. While IFX is a treatment of choice in steroid-refractory and steroid-dependent patients, other options that can be used for these patients as a rescue therapy include anti-TNF-α monoclonal antibodies (adalimumab and golimumab), calcineurin inhibitors (tacrolimus and cyclosporine), anti-$\alpha_4\beta_7$ integrin (vedolizumab), and cytapheresis [10, 11]. No consensus on a strategy for corticosteroid-refractory or corticosteroid-dependent moderate-to-severe UC patients has been reached, since evidence directly comparing these options does not exist, except for a head-to-head comparison showing no difference between IFX and cyclosporine [12]. In addition, no definitive predictive factor has been reported to help choose a given treatment option.

Monitoring serum concentrations of IFX has been considered useful in evaluating the mechanism of loss of response to IFX. Afif et al. reported that low drug levels drawn 4 or 8 weeks after a scheduled infusion every 8 weeks predict loss of response after initial success with IFX [13]. A therapeutic algorithm for patients with secondary failure based on serum IFX trough levels (TLs) and the development of antibodies (ATIs) against IFX has been suggested to help determine an appropriate treatment intervention [13, 14].

Acute severe flares are one of the most critical features of UC. They may require hospitalization and surgery. Because decisions on whether a treatment should be continued or switched to the next option are often made with a lack of data about possible outcomes, and may result in necessary surgery being delayed, the earliest possible evaluation of the response to treatment is crucial. In clinical practice, judging short- and long-term efficacy soon after the initial infusion of IFX is always challenging: some patients gradually respond over time after multiple infusions, whereas others demonstrate a transient response followed by a subsequent loss of efficacy. Thus, the availability of an early and accurate predictor of outcome would be of particular value.

Here, as part of a phase 3 trial of IFX efficacy and safety in UC, we examined the accuracy of TL measurements as a predictive factor of therapeutic response early in IFX treatment.

Methods

Patients

This randomized, double-blind, placebo-controlled study (Japic CTI-060298) was conducted between July 2006 and December 2008 among 208 patients at 67 medical institutions in Japan. The protocol was approved by the institutional review board at each medical institution. All patients gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

All eligible patients had an established diagnosis of UC and had been screened for tuberculosis by the skin test and chest radiography. Endoscopy was performed during screening to confirm disease activity. Eligible patients had no evidence of tuberculosis and had active UC with a Mayo score [15] of 6–12 points and an endoscopic subscore of $\geq$2. Documentation of one or more previous treatment failures was required at enrollment, namely, we enrolled patients with no response to immunomodulators (azathioprine and 6-mercaptopurine) that were started at least 12 weeks before enrollment and maintained at a stable dose from at least 4 weeks before enrollment or that were administered for at least 12 weeks within the preceding 5 years; patients who could not tolerate immunomodulators within the preceding 5 years; patients who had had no response to oral corticosteroids started at least 2 weeks before enrollment at 20 mg/day or more and maintained at a stable dose (corticosteroid-refractory); and patients who showed no response to corticosteroids (equivalent prednisolone 40 mg/day or more, for at least 2 weeks orally, or for at least a week intravenously), worsening of UC with tapering of corticosteroids (corticosteroid-dependent), or corticosteroid intolerance within the previous 18 months.

We excluded patients with a history of recent bowel surgery; bowel complications such as stricture, fistula, or dysplasia; or treatment with other biologics, methotrexate, calcineurin inhibitors, or cytapheresis within the previous 18 months because of the possible influence of these treatments on the efficacy of IFX. Patients with serious medical conditions such as chronic heart failure or latent infectious diseases (hepatitis C, human immunodeficiency virus infection, or any other chronic infectious disease) were also excluded.

Study design

Eligible patients were randomly assigned in a 1:1 ratio to receive an intravenous infusion of IFX at a dose of 5 mg/kg or placebo at weeks 0, 2, and 6 (Fig. 1a). Randomization was performed centrally with the use of computer-generated randomization schedules stratified according to the investigational site and concomitant use or nonuse of corticosteroids (prednisolone equivalent of 0, <20, or ≥20 mg/day). Patients with a significantly lower Mayo score at week 8 (defined as a decrease in the total Mayo baseline score of at least 3 points and at least 30 %, with an accompanying decrease in the rectal bleeding subscore of at least 1 point or an absolute subscore of 0 or 1, i.e., 8-week responders) then received IFX or placebo at weeks 14 and 22. Conversely, 8-week non-responders were discontinued from IFX or placebo treatment. Eight-week responders were followed through week 38. Eight-week non-responders and other patients who discontinued treatment with IFX or placebo were followed until 16 weeks after the last administration.

Permitted concomitant medications for UC included corticosteroids (started at least 2 weeks before sigmoidoscopy at enrollment and maintained at a stable dose), oral aminosalicylates (started at least 3 weeks before enrollment and maintained at a stable dose), azathioprine and 6-mercaptopurine (started at least 12 weeks before enrollment and maintained at a stable dose from at least 4 weeks before enrollment). Doses of concomitant medications remained constant throughout the study except for corticosteroids, which were tapered by 5 mg/week after week 8 until a dose of 20 mg/day was reached and then by 2.5 mg/week.

End points

The primary end point was a clinical response at week 8. Secondary end points were clinical remission or MH

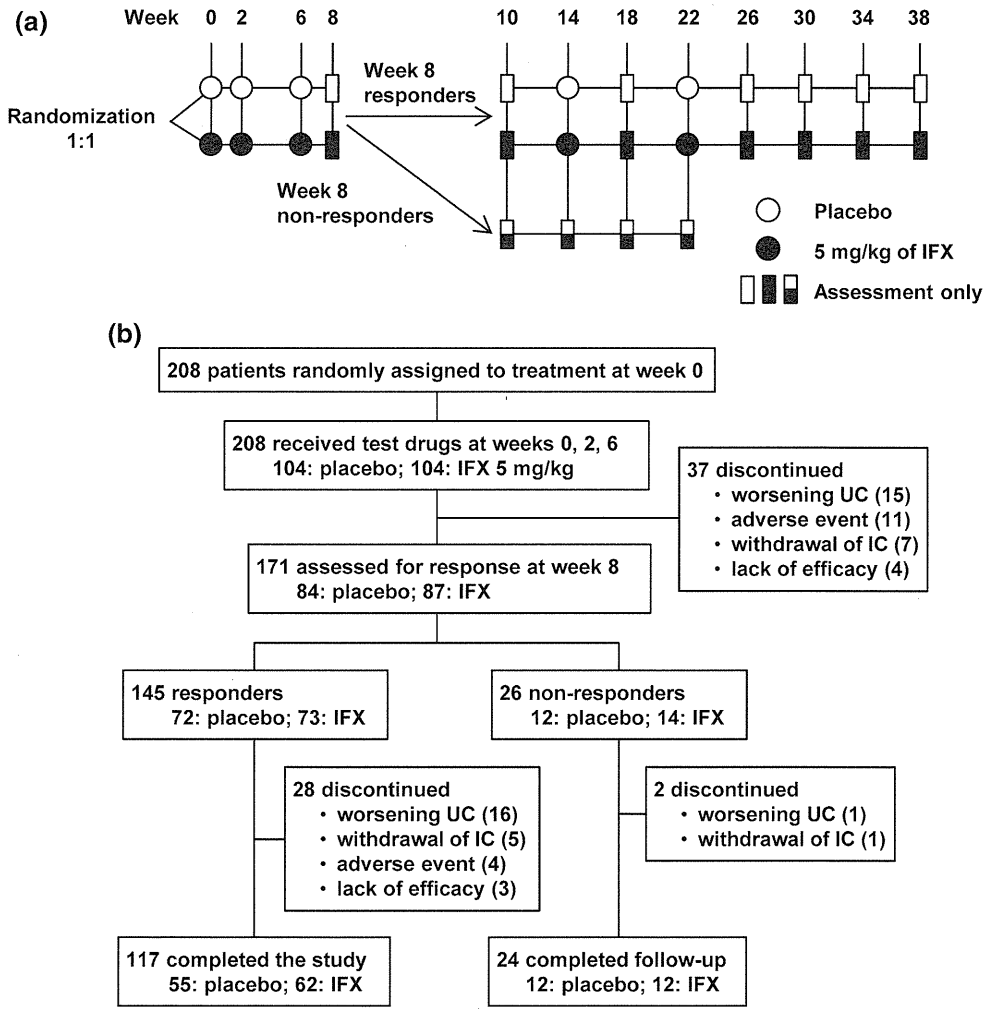

Fig. 1 Study schema (**a**), patient disposition (**b**). *IFX* infliximab; *UC* ulcerative colitis; *IC* informed consent

response at week 8 as well as clinical response or clinical remission at week 30. The Mayo score was determined at weeks 0, 8, and 30. Clinical remission was defined as a total Mayo score of 2 points or lower, with no individual subscore exceeding 1 point. MH was defined as an endoscopic subscore of 0 or 1. The clinical activity index (CAI) [16] was determined at all visits. CAI remission was defined as a CAI score of 4 or lower.

TL at weeks 0, 2, 6, and every 8 weeks thereafter and ATI at weeks 0, 14, and 30 were measured using an enzyme-linked immunosorbent assay [17] (Mitsubishi Tanabe Pharma Corp., Osaka, Japan).

Statistical analysis

Efficacy was assessed in the full analysis set. Patients who took prohibited medication because of worsening UC (lack of efficacy or loss of response to the study medication), who discontinued the study medication because of worsening UC, including 8-week non-responders, or who underwent colectomy or colostomy were not considered to have had a clinical response, clinical remission, or MH, and their post-procedure CAI score was used as the baseline value from the time of the procedure onward. For other patients who withdrew prematurely, the last observation was carried forward. Statistical tests were two-sided, with $p \leq 0.05$ considered to indicate statistical significance.

Demographic and baseline characteristics were compared using Fisher's exact test, Student's t test, or the Wilcoxon rank test. Proportions of patients with a clinical response, clinical remission, and MH were compared using logistic regression analysis [explanatory variables: treatment group, corticosteroid use (except for analysis in severe cases)].

Post hoc analysis

We conducted post hoc analysis in 82 patients who were treated with IFX at weeks 0, 2, and 6 and evaluated until week 14. The ratio of TL to CAI (TL/CAI) was calculated using the CAI value plus 1, because CAI scores include 0. Between-group differences in the TL and CAI remission rates were calculated with the Wilcoxon rank test and Fisher's exact test, respectively. Receiver-operating characteristic (ROC) curve analysis was performed to assess the performance of the week 2 TL or TL/CAI for predicting CAI remission at week 14. Predictive factors in baseline characteristics as well as clinical and laboratory parameters identifying patients with CAI remission at week 14 were analyzed with logistic regression analysis. The cutoff values for age, body weight, TL, and TL/CAI used median values, while those for other end points used their reference values. Multiple logistic regression analysis was performed to identify independent predictors of 14-week CAI remission. The explanatory variables used in these multiple analyses were sex, age <39.5 years, weight <56.0 kg, smoker, colonic area involved, oral corticosteroid use, oral 5-aminosalicylate use, immunomodulator use, corticosteroid-refractory disease, corticosteroid-dependent disease, CAI remission at week 2, the week 2 TL (first TL) $\geq$ the median value, and the first TL/CAI $\geq$ the median value. Variables were chosen using stepwise backward selection to minimize the Bayesian information criterion.

Mitsubishi Tanabe Pharma Corp. sponsored this clinical trial and was responsible for the collection and analysis of data.

Results

Clinical outcome

Patient disposition is shown in Fig. 1b. There were no significant differences in backgrounds between the IFX and placebo groups (Table 1). At week 8, the clinical response rate, the primary end point, was significantly higher in patients who received IFX than in those who received placebo ($p = 0.005$; Table 2). The clinical remission rate showed marginal significance ($p = 0.054$), whereas MH was significantly more frequent with IFX than placebo ($p = 0.006$). Results of clinical response, clinical remission, and MH at week 30 are shown in Table 2. Response rate at week 8 in acute severe cases ($n = 18$, Mayo score $\geq$11) was similar to that in the entire study population, although it did not reach statistical significance because of the limited number of patients [54.5 % (6/11) in the IFX group versus 28.6 % (2/7) in the placebo group, $p = 0.287$].

Since this study was conducted as part of a nationwide phase 3 trial in Japan, safety profiles were also carefully calculated for all patients. There was no difference in the incidence of any adverse events, including serious infections and infusion reactions, during the short-term as well as the entire follow-up period in this trial (Table 3).

Clinical course of patients treated with IFX

We next assessed the clinical course of 82 patients who received the initial three infusions at weeks 0, 2, and 6 and evaluated them until week 14 to focus on the initial response to induction therapy with IFX, using CAI determined at all visits. Among these 82 patients, 37 (45 %) achieved CAI remission at week 2, but 8 (22 %) lost their response by week 14 (Fig. 2a). In contrast, 17 (38 %) patients who were not in CAI remission at week 2 improved and achieved CAI remission at week 14. Interestingly, most patients who achieved CAI remission

Table 1 Baseline characteristics of patients

Characteristic	Placebo ($n = 104$)	IFX[a] ($n = 104$)	p
Male sex n (%)	67 (64.4)	66 (63.5)	1.000[c]
Age (years)	37.8 ± 12.9	40.0 ± 12.7	0.220[d]
Median (IQR)[b]	36.0 (28.0, 47.0)	39.5 (29.5, 49.0)	
Weight (kg)	60.3 ± 11.6	57.6 ± 12.7	0.117[d]
Median (IQR)	59.0 (52.0, 69.0)	56.0 (49.0, 64.0)	
Smoking n (%)	6 (5.8)	9 (8.7)	0.593[c]
Duration of disease (years)	7.1 ± 6.6	8.1 ± 7.2	
Median (IQR)	4.7 (2.5, 9.2)	6.5 (3.5, 11.0)	0.133[e]
Colonic area involved			
Left side n (%)	20 (19.2)	21 (20.2)	1.000[c]
Extensive n (%)	84 (80.8)	83 (79.8)	
Concomitant medications n (%)			
Corticosteroids (oral)	69 (66.3)	68 (65.4)	1.000[c]
5-Aminosalicylates (oral)	70 (67.3)	77 (74.0)	0.361[c]
Immunomodulators	49 (47.1)	50 (48.1)	1.000[c]
Azathioprine	34 (32.7)	38 (36.5)	0.662[c]
6-Mercaptopurine	15 (14.4)	12 (11.5)	0.681[c]
Corticosteroid-refractory disease n (%)	20 (19.2)	20 (19.2)	1.000[c]
Corticosteroid-dependent disease n (%)	52 (50.0)	44 (42.3)	0.330[c]
Mayo score	8.5 ± 1.4	8.6 ± 1.4	0.435[d]
Median (IQR)	9.0 (7.0, 9.5)	9.0 (8.0, 9.0)	
Clinical activity index[f]	8.2 ± 2.4	8.4 ± 2.6	0.502[d]
Median (IQR)	8.0 (6.0, 10.0)	8.0 (7.0, 10.0)	
C-reactive protein mg/dl	0.7 ± 1.1	1.0 ± 1.5	
Median (IQR)	0.4 (0.1, 0.9)	0.5 (0.1, 1.0)	0.092[e]
Albumin g/dl	3.9 ± 0.4	3.9 ± 0.4	
Median (IQR)	3.9 (3.7, 4.2)	3.9 (3.7, 4.1)	0.400[e]

[a] IFX infliximab

[b] IQR interquartile range

[c] Fisher's exact test

[d] Student's t-test

[e] Wilcoxon rank test

[f] Placebo, $n = 104$, infliximab, $n = 103$

Data are shown as the mean ± SD except where indicated otherwise

at week 14 remained in remission until week 30, more frequently than the week 8 CAI remitters. Week 14 therefore appeared to be an appropriate time point to define the primary clinical response for the following analyses. TL was significantly higher in patients who were in CAI remission than in those who were not at each time point after the second infusion at week 6 (Fig. 2b).

Assessment of TL

Early prediction of clinical efficacy is especially important for the treatment of acutely ill patients. Therefore, we conducted a post hoc analysis to examine the potential usefulness of TL at the early time point for prediction of future outcome.

Interestingly, an association between week 2 TL and clinical efficacy showed a tendency to be stronger at the later time point (Fig. 3a) [the optimal cutoff value for predicting 14-week CAI remission: 21.3 μg/ml; 0.61 sensitivity, 0.69 specificity, 0.72 positive predictive value, and 0.58 negative predictive value; area under the curve: 0.65, 95 % confidence interval (CI): 0.52–0.77]. The correlation of TL with MH was also evaluated, since MH is considered an important predictive factor for long-term outcome. Similar to clinical efficacy, the first TL showed a significant correlation with MH at week 30 but not at week 8 (Fig. 3b).

Table 2 Summary of efficacy results

Characteristics	Placebo ($n = 104$)	IFX[a] ($n = 104$)	p[b]
Clinical response *n* (%)			
Week 8	37 (35.6)	57 (54.8)	0.005
Week 30	33 (31.7)	48 (46.2)	0.033
Clinical remission *n* (%)			
Week 8	11 (10.6)	21 (20.2)	0.054
Week 30	17 (16.3)	22 (21.2)	0.373
Mucosal healing *n* (%)			
Week 8	29 (27.9)	48 (46.2)	0.006
Week 30	30 (28.8)	43 (41.3)	0.057

[a] IFX, infliximab

[b] Logistic regression analysis (explanatory variable: treatment group, corticosteroid use)

Usefulness of the first TL in predicting clinical outcome

We screened for the predictive values of multiple clinical parameters, including background and 2-week clinical parameters. Results showed that there was no patient background factor, including the severity and laboratory data, that demonstrated a significant association with clinical outcome at week 14 (Table 4). However, CAI remission at 2 weeks was significantly associated with the 14-week CAI remission, in addition to the first TL. This suggests that patients already in remission at week 2 are likely to remain in remission at week 14. However, this is not useful in identifying patients who gain/lose their response to IFX after week 2. We therefore evaluated the first TL/CAI and found that this ratio also correlated with 14-week CAI remission (Table 4) (the optimal cutoff value for predicting 14-week CAI remission: 4.3; 0.61 sensitivity, 0.81 specificity, 0.80 positive predictive value, and 0.62 negative predictive value; area under the curve: 0.76, 95 % CI: 0.65–0.86). Accordingly, although it did not reach statistical significance because of the limited sample size, the week 14 CAI remission rate in 2-week non-remitters (subgroup of delayed response) with the first TL/CAI of $\geq$4.3 tended to be higher than in those with the first TL/CAI <4.3 (50 % versus 36 %). Furthermore, the week 14 CAI non-remission rate in 2-week remitters (subgroup of loss of response) with the first TL/CAI of $\geq$4.3 tended to be lower than in those with the first TL/CAI <4.3 (14 % versus 44 %). In multiple logistic analysis, the first TL/CAI (odds ratio 8.07, 95 % confidence interval 2.84–27.07, $p < 0.001$) and 5-aminosalicylate use (odds ratio 3.98, 95 % confidence interval 1.29–14.26, $p = 0.016$) were identified as independent factors that correlated with 14-week CAI remission. The first TL/CAI was significantly associated with 30-week MH as well as with 14-week CAI remission (Fig. 3c, d).

Discussion

We assessed the predictive value of the first TL at week 2 for clinical outcome as part of a phase 3 randomized controlled trial of IFX in UC. Results showed the utility of the week 2 first TL as a predictive factor for both short- and long-term outcomes in these patients.

Previous studies reported that low serum albumin, absence of concomitant immunomodulator use, and severe disease were associated with poor response to IFX [18–21]. However, in our study population, neither these nor any other baseline characteristics were associated with clinical outcome, suggesting that patient background, including severity and laboratory data, is not useful in predicting clinical outcome before the start of therapy. Therefore,

Table 3 Safety profiles

	By week 14		Entire period	
	Placebo ($n = 104$)	IFX[a] ($n = 104$)	Placebo ($n = 104$)	IFX ($n = 104$)
Follow-up days, mean (range)	98.3 (92–106)	98.1 (66–106)	214.1 (111–274)	223.2 (66–274)
Mean doses *n*	2.7	2.9	3.9	4.2
Any adverse event	86 (82.7)	85 (81.7)	94 (90.4)	100 (96.2)
Infections	35 (33.7)	33 (31.7)	51 (49.0)	62 (59.6)
Infusion reactions	9 (8.7)	11 (10.6)	11 (10.6)	16 (15.4)
Adverse events leading to discontinuation of study drug	8 (7.7)	5 (4.8)	8 (7.7)	7 (6.7)
Any serious adverse event	13 (12.5)	9 (8.7)	19 (18.3)	18 (17.3)
Worsening ulcerative colitis	11 (10.6)	8 (7.7)	18 (17.3)	16 (15.4)
Serious infections	2 (1.9)	1 (1.0)	2 (1.9)	1 (1.0)
Serious infusion reactions	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[a] IFX, infliximab

Data are shown as the number of patients (%) except where indicated otherwise

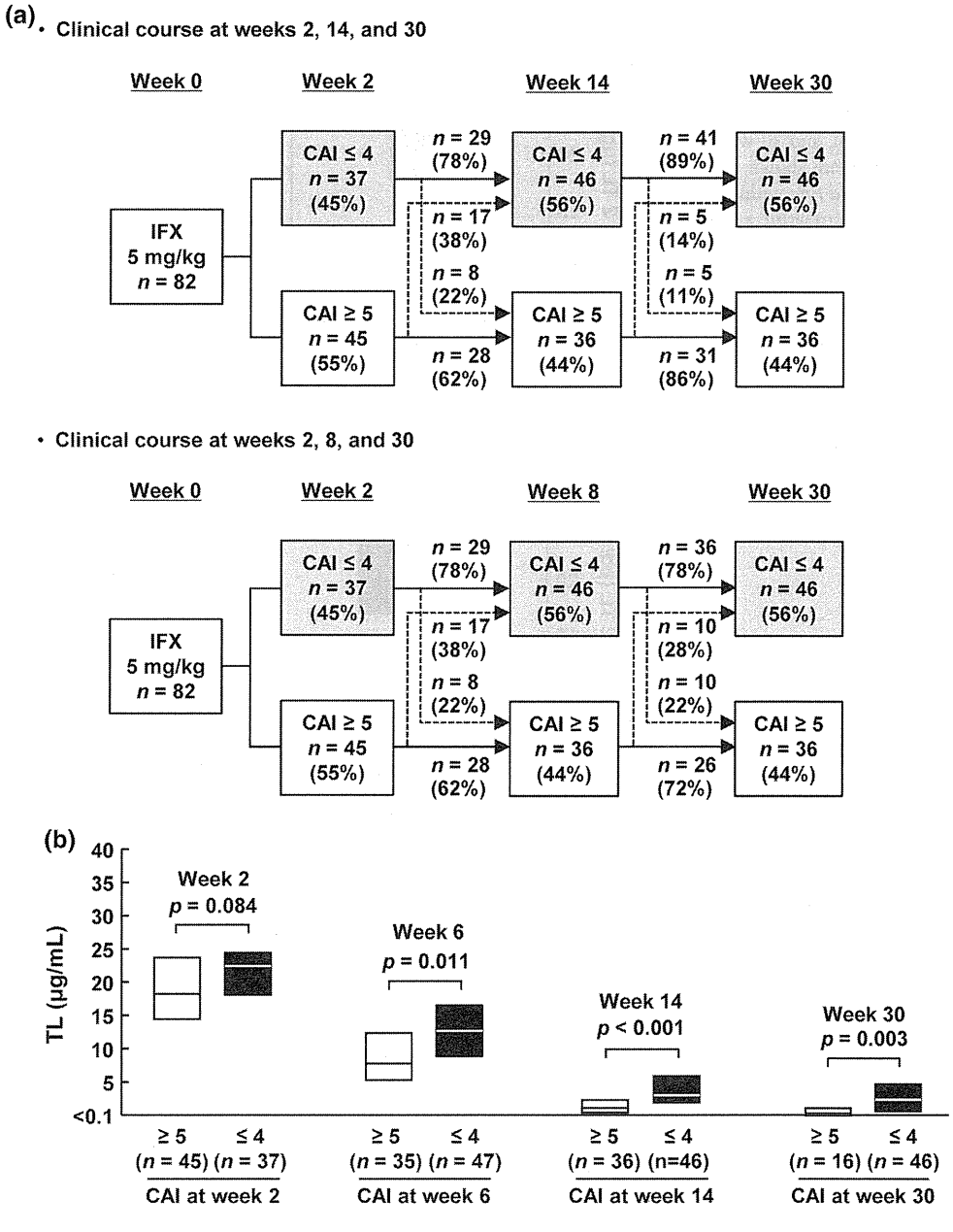

Fig. 2 Clinical course and TL of patients treated with IFX. **a** Clinical course of patients who achieved CAI remission. **b** TL according to clinical efficacy. Results are shown as median (interquartile range). Statistical differences were calculated with the Wilcoxon rank test. *TL* serum infliximab (IFX) trough level; *CAI* clinical activity index

early detection of response to IFX is critical for the management of UC, especially in acute severe patients (e.g., Mayo score ≥11 or CAI ≥12), whose clinical outcome was comparable with that in the moderately active patients. It has been reported that TL is associated with the clinical status at each time point in patients under maintenance therapy [22, 23], but its predictive role for future outcomes during induction therapy has not been studied. Therefore, we hypothesized that the first TL may be useful and found that the first TL significantly correlates with 14-week primary response. Interestingly, the correlation between the first TL and clinical outcome was seen even at week 30, suggesting that the 30-week clinical efficacy can be predicted after as little as 2 weeks. Furthermore, we also found that the first TL correlated with the achievement of MH at 30 weeks. Post hoc analysis was conducted in 82 patients who received treatment at weeks 0, 2, and 6 and were evaluated until week 14. However, even after including the 11 patients who discontinued the therapy because of worsening UC or lack of efficacy by week 14, the first TL showed significant correlations with 14-week remission and 30-week MH (data not shown). Although we did not conduct a long-term follow-up in this study, our results suggest that the first TL may predict not only the short-term, but also long-term outcome of subsequent IFX maintenance therapy, since MH is reported to be associated