ORIGINAL RESEARCH

Addition of azathioprine to the switch of anti-TNF in patients with IBD in clinical relapse with undetectable anti-TNF trough levels and antidrug antibodies: a prospective randomised trial

Xavier Roblin,[1] Nicolas Williet,[1] Gilles Boschetti,[2] Jean-Marc Phelip,[1] Emilie Del Tedesco,[1] Anne-Emmanuelle Berger,[3] Philippe Vedrines,[4] Gerard Duru,[5] Laurent Peyrin-Biroulet ,[6,7] Stéphane Nancey,[2] Bernard Flourie,[2] Stephane Paul [3]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2019-319758).

For numbered affiliations see end of article.

Correspondence to
Professor Stephane Paul, Department of Immunology, CIC 1408, GIMAP, CHU Saint-Étienne, Saint-Etienne, Rhône-Alpes 42055, France; stephane.paul@chu-st-etienne.fr

Received 30 August 2019
Revised 10 January 2020
Accepted 13 January 2020

To cite: Roblin X, Williet N, Boschetti G, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2019-319758

ABSTRACT

Objectives In patients with IBD experiencing an immune-mediated loss of response (LOR) to antitumour necrosis factor (anti-TNF), algorithms recommend a switch of anti-TNF without immunosuppressive drug. The aim of our study was to compare in these patients two strategies: either switch to a second anti-TNF alone or with addition of azathioprine (AZA). After randomisation outcomes (time to clinical and pharmacokinetic failure) were compared between the two groups during a 2-year follow-up period.

Design Consecutive IBD patients in immune-mediated LOR to a first optimised anti-TNF given in monotherapy were randomised to receive either AZA or nothing with induction by a second anti-TNF in both arms. Clinical failure was defined for Crohn's disease (CD) as a Harvey-Bradshaw index ≥5 associated with a faecal calprotectin level >250 µg/g stool and for UC as a Mayo score >5 with endoscopic subscore >1 or as the occurrence of adverse events requiring to stop treatment. Unfavourable pharmacokinetics of the second anti-TNF were defined by the appearance of undetectable trough levels of anti-TNF with high antibodies (drug-sensitive assay) or by that of antibodies (drug-tolerant assay).

Results Ninety patients (48 CDs) were included, and 45 of them received AZA after randomisation. The second anti-TNF was adalimumab or infliximab in 40 and 50 patients, respectively. Rates of clinical failure and occurrence of unfavourable pharmacokinetics were higher in monotherapy compared with combination therapy (p<0.001; median time of clinical failure since randomisation 18 vs >24 months). At 24 months, survival rates without clinical failure and without appearance of unfavourable pharmacokinetics were respectively 22 versus 77% and 22% versus 78% (p<0.001 for both) in monotherapy versus combination therapy. Only the use of combination therapy was associated with favourable outcomes after anti-TNF switch.

Conclusion In case of immune-mediated LOR to a first anti-TNF, AZA should be associated with the second anti-TNF.

Trial registration number 03580876.

Significance of this study

What is already known on this subject?

- Loss of response due to immunogenicity (low or undetectable trough levels of antitumour necrosis factor (anti-TNF) with high levels of antidrug antibodies) accounts for ≈20% of therapeutic escapes under anti-TNF.
- In patients with an immune-mediated pharmacokinetic failure, algorithms based on therapeutic drug monitoring propose a switch of anti-TNF without defining clearly the interest of adding an immunosuppressive drug.
- Different studies have shown that the addition of an immunosuppressive drug in case of an immune-mediated pharmacokinetic failure may result in the restoration of favourable pharmacokinetics and clinical response.

What are the new findings?

- Clinical and pharmacokinetic evolution was significantly more favourable after a switch to a second anti-TNF with azathioprine compared with a switch without azathioprine in a randomised prospective trial.
- Only the use of combination therapy was significantly associated with favourable outcomes after anti-TNF switch.

How might it impact on clinical practice in the foreseeable future?

- In case of immune-mediated loss of response to a first anti-TNF, azathioprine should be used in combination with the second anti-TNF.

INTRODUCTION

Under antitumour necrosis factor (anti-TNF), loss of response is frequent, estimated at 13% per year for infliximab (IFX)[1] and 20% per year for adalimumab (ADA).[2] A large Spanish study showed that the cumulative incidence of loss of response at 5 years was 45% after a second anti-TNF line and 38% after a third anti-TNF line.[3] Loss of response due to immunogenicity (low or undetectable trough levels of anti-TNF with high levels of antidrug antibodies)

accounts for 20% of therapeutic escapes under anti-TNF.[4] In these patients, the current algorithms based on therapeutic drug monitoring (TDM) propose a switch of anti-TNF without defining clearly the interest of adding an immunosuppressive drug.[5] In a recent study, we showed that the pharmacokinetics of the first anti-TNF in the case of loss of response influenced the pharmacokinetics of the second anti-TNF.[6] Thus, patients with an immunogenic failure to a first anti-TNF developed within 2 years of follow-up an immunogenic failure to the second anti-TNF prescribed as monotherapy in 85% of cases.

Antidrug antibody induction is more frequent under IFX than under ADA.[7] In the large Personalising Anti-TNF Therapy in Crohns Disease (PANTS) study,[8] the rate of antibodies to IFX (ATI) and to ADA (ATA) measured with a drug-tolerant immunoassay was 62.8% and 28.5% at 1 year, respectively. In this study, immunomodulator use was the major protective factor against immunogenicity, with similar effect sizes for IFX and ADA. Other cohort studies have shown that the addition of an immunosuppressive drug in case of an immune-mediated pharmacokinetic failure resulted in the restoration of favourable pharmacokinetics and clinical response in nearly 50% of cases.[9 10] In view of these results, the addition of an immunosuppressive drug when switching to another anti-TNF agent in case of immunogenic failure seems to be the more appropriate approach.

The aim of our study was to compare two strategies in patients with IBD who experienced an immune-mediated pharmacokinetic failure with a first anti-TNF given at an optimised dose: either switch to a second anti-TNF agent alone or switch to a second anti-TNF with addition of azathioprine (AZA). After randomisation, rates of clinical and immunogenic failure and of adverse events were compared between the two groups of patients during a 2-year follow-up period.

Patients and methods

It was a two centres (university hospitals of Lyon and Saint-Etienne), randomised, open-label and prospective trial (Clinical Trial Number: 03580876). After informed and signed consent, all consecutive patients presenting a clinical and immunogenic failure to an optimised dose of a first anti-TNF (ADA 40 mg each week or IFX 10 mg/kg every 8 weeks) were randomised to undergo a switch to a second anti-TNF alone (switch to ADA in case of failure under IFX and switch to IFX in case of failure under ADA) or a switch to this second anti-TNF with addition of AZA (2–2.5 mg/kg/day) (figure 1).

Included patients with IBD were in clinical failure under a first anti-TNF given alone at optimised dose. Clinical failure was defined as a Harvey-Bradshaw index (HBI) ≥5 associated with

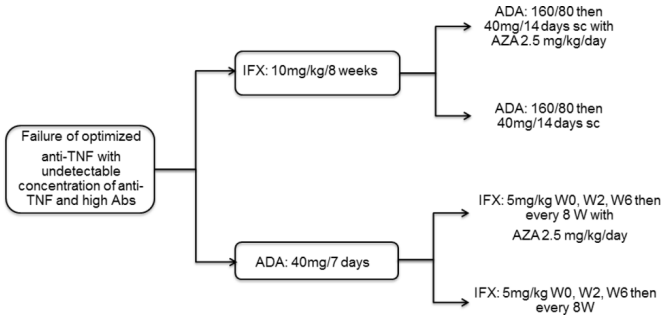

Figure 1 Design of the randomised prospective study. Abs, antibodies; ADA, adalimumab; AZA, azathioprine; IFX, infliximab; TNF, tumour necrosis factor; W, week.

faecal calprotectin levels >250 µg/g stools for Crohn's disease (CD) and as a Mayo score >5 with an endoscopic subscore >1 for UC. Patients must be treated with an optimised dose for at least 4 months before the inclusion and on monotherapy for at least 6 months before the inclusion. ADA was usually prescribed as monotherapy and, as previously suggested by Van Assche *et al*,[11] immunosuppressant drug was stopped after 6–12 months of combination therapy with IFX. Included patients presented also an immune-mediated pharmacokinetic failure to the first anti-TNF defined by undetectable trough levels of IFX (TLI) or of ADA (TLA) with high antibody titres (≥20 ng/mL) determined using a drug-sensitive ELISA test (Theradiag, Marne-la-Vallée, France) from two consecutive samples separated by at least 1 week. In clinical practice, we collected blood samples in case of clinical failure for TDM measurements. Therefore, 100 patients with clinical and pharmacokinetic failure were screened before randomisation. One week before randomisation, a second TDM measurement was performed, and results were obtained within the same week. If this assessment confirmed the immunogenic failure, patients were definitively included. No patient was excluded due to transient antibodies.

Patients with age below 18 years old, presenting an undetermined colitis, pregnant women, CD patients with a predominant perianal disease or ostomy and patients intolerant or contraindicated to AZA were not screened. Patients with a primary non-response to the first anti-TNF were not included in our study. Patients were randomised into two parallel groups with randomisation balanced by blocks: group monotherapy (switch to the second anti-TNF) and group combination therapy (switch to the second anti-TNF with addition of AZA). The randomisation was not stratified and was centrally performed by an interactive web response. After randomisation, an induction regimen was performed for all patients followed with a maintenance dose (ADA: 160 mg at week 0, 80 mg at week 2 and then 40 mg every other week; IFX: 5 mg/kg at week 0, week 2 and week 6 and then every 8 weeks). After randomisation, patients were followed for 24 months or less in case of failure. The clinical activity (HBI for CD and partial Mayo score for UC) was calculated before each infusion of IFX and every 8 weeks for ADA. Anti-TNF pharmacokinetic assessments were performed just before infusion of IFX or injection of ADA at 6, 12, 18 and 24 months since randomisation in the absence of clinical failure or at time of clinical failure during the follow-up. All pharmacokinetic results were blinded between clinical and immunologic units until the end of the study.

Clinical failure was defined for CD as an HBI ≥5 with faecal calprotectin levels >250 µg/g stools and for UC as a total Mayo score >5 with an endoscopic subscore >1. Clinical failure was also defined when adverse events occurred and required to stop treatment. Using a drug-sensitive assay, pharmacokinetic failure was defined as the development of unfavourable pharmacokinetics of the second anti-TNF, that is, undetectable trough levels of the drug (undetectable TLI or TLA) with high antibodies (ATI or ATA ≥20 ng/mL). Using a drug-tolerant assay based on a method previously described by Ben-Horin *et al*,[12] pharmacokinetic failure was considered when positive antidrug antibodies were isolated (>2 µg/mL Eq). Transient antidrug antibodies were defined as antibodies that appeared during the course of anti-TNF therapy, were not associated with clinical worsening and disappeared between two consecutive measurements.

TLI, TLA, ATI and ATA concentrations were all measured using the Lisa-Tracker Premium Infliximab and Adalimumab ELISA kits (Theradiag) just before infusion of IFX or injection of ADA. This drug-sensitive assay has been developed to reduce low-affinity

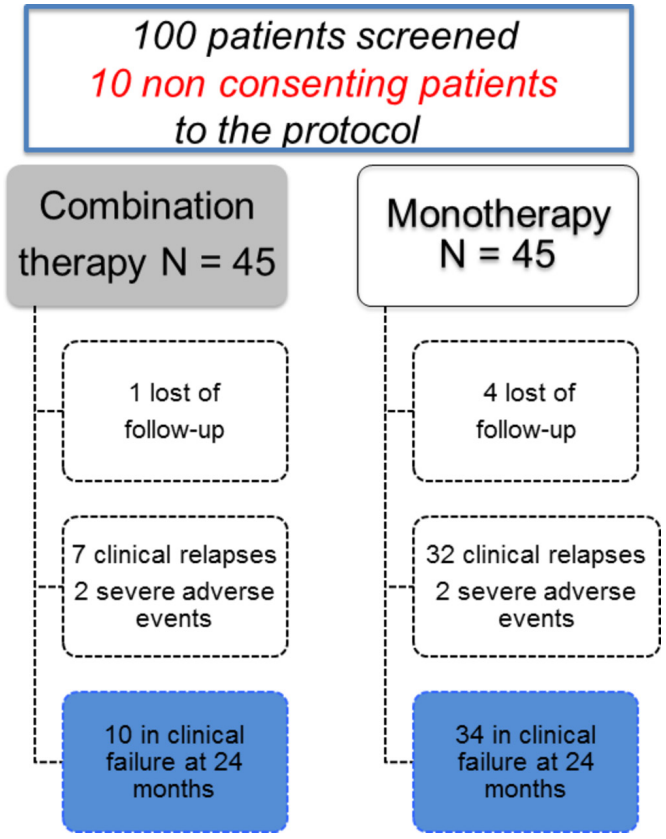

Figure 2 Flow chart of the study.

binding of immune complexes or interfering molecules such as the rheumatoid factor. The use of specific buffers for both binding and washing steps allows a very efficient capture of free molecules. ATI and ATA were also measured using a drug-tolerant assay. Briefly, they were evaluated by a previously described drug-tolerant ELISA using antihuman lambda chain for the detection of antidrug antibodies.[12] Calprotectin was measured in stools using the fCAL turbo assay (Buhlmann, Basel, Switzerland).

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS V.20.0.0. Continuous variables were expressed as mean and SD or median with IQR, and categorical variables were expressed as percentage. The $\chi 2$ test and the Mann-Whitney test were used to compare categorical and quantitative variables as appropriate. Rates of failure were compared using Kaplan-Meier curves and log-rank statistics. $P<0.05$ was considered statistically significant. Factors associated with failure were isolated using Cox regression model. Calculation of sample size was performed with assumed clinical failure of about 35% for the combination therapy group and 70% for the monotherapy group. Under these assumptions and with a hypothesis of 10% dropouts, 45 patients per group with a total of 90 patients would provide an 80% of power at a one-sided alpha-level of 0.05 to detect difference in the clinical endpoint. When we compared rates of failure between the two groups of patients at intermediate times, we used the worst hypothesis.[13 14] This imputation was not applied to the time to failure but only applied to the rate of failure. Patients lost to follow-up under monotherapy before the intermediate time were not considered in clinical failure. Conversely, under combination of treatment, patients lost to follow-up were defined as in clinical failure.

RESULTS

Characteristics of patients

Ninety patients (mean age: 39.5 years; sex ratio M/F: 0.95; 48 CD) were included in this randomised trial, 45 in each group (table 1 and figure 2). The mean duration of disease was 3.5 years and 63% of patients had been previously treated with an immunosuppressive drug. Forty patients switched to ADA and 50 to IFX. At the inclusion, the median concentrations of ATA and ATI were, respectively, 55 ng/mL and 80 ng/mL using a drug-sensitive assay and 36 (40%) patients presented very high levels of antidrug antibodies (≥100 ng/mL). The two groups of

Table 1 Characteristics of included patients

	Total	Monotherapy	Combination therapy	P values
Number	90	45	45	0.9
Age (years) (mean, SD)	39.5 (14.5)	39	40.5	0.7
Sex ratio M/F	0.95	1	0.9	0.9
CD, N	48	23	25	0.8
Phenotype	L1: 25, L2: 11, L3: 12; B1: 30, B2: 7, B3: 11	L1:12, L2: 6, L3: 5 B1: 14, B2: 3, B3: 6	L1: 13, L2: 5, L3: 7 B1: 16, B2: 4, B3: 5	0.5 0.3
Perianal (p)	p: 10	p: 5	p: 5	0.9
History of intestinal resection (N)	12	5	7	0.8
HBI (mean, SD)	10.5 (2.5)	9.8 (1.7)	10.8 (2.6)	0.6
UC, N	42	22	20	0.9
Phenotype	E1: 6, E2: 24, E3: 12	E1:3, E2:14, E3: 5	E1:3, E2:10, E3 :7	0.8
Mayo score (mean, SD)	8.5 (2.2)	9.2 (1.4)	8.3 (2.3)	0.6
Duration of disease (years) (mean, SD)	3.5 (1.8)	3.4	3.6	0.9
Active smoking, N	39	22	17	0.3
Previous immunosuppressors, N (%)	57 (63.3)	29 (64.2)	28 (62.2)	0.8
ADA switch, N	40	24	16	0.5
IFX switch, N	50	21	29	0.8
C reactive protein (mg/L) (median (IQR))	15 (2–22)	13	16	0.4
ATA (ng/mL) (median (IQR))	55 (20–120)	52	59	0.7
ATI (ng/mL) (median (IQR))	80 (30–200)	94	75	0.5

ADA, adalimumab; ATA, antibodies to adalimumab; ATI, antibodies to infliximab; CD, Crohn's disease; HBI, Harvey-Bradshaw Index; IFX, infliximab; TNF, tumour necrosis factor.

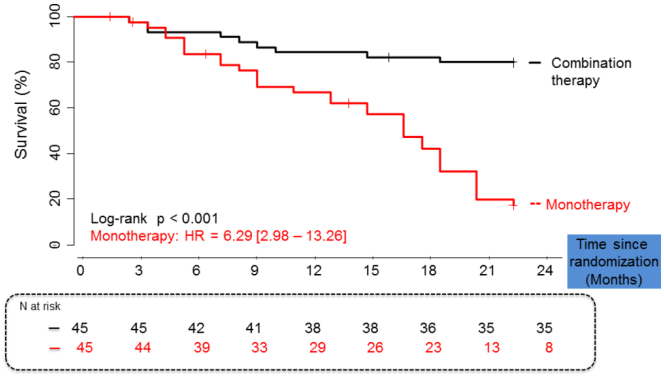

Figure 3 Evolution without clinical failure.

patients (monotherapy vs combination of treatment) were very similar according to all parameters recorded in this study. Five patients were lost to follow-up during the 2year follow-up: one in the group combination of treatment and four in the group monotherapy.

Clinical failure since randomisation during the follow-up

After the induction at week 14, no patient presented clinical failure. Later, rates of clinical failure were significantly higher in the group monotherapy (log-rank test p<0.001 (HR=6.29 (95% CI 2.98 to 13.26)) (figure 3). The results were similar whatever the anti-TNF (IFX or ADA) used after the switch (figure 4) in monotherapy (ADA vs IFX; p=0.46) or in combination of treatment (ADA+AZA vs IFX+AZA; p=0.39). Combination of treatment decreased significantly the rates of clinical failure compared with monotherapy (ADA+AZA: HR=0.11 (95% CI 0.02 to 0.47); p=0.003 and IFX+AZA: HR=0.22 (95% CI 0.09 to 0.52); p<0.001). Evolution without clinical failure was significantly different between the two groups of patients from 15 months (p=0.04) but was not significantly different at 6 months and 12 months (p=0.6 and p=0.2, respectively). At 6 months, the percentages of patients without clinical failure were 89% and 79% under combination of treatment and monotherapy, respectively (p=0.6). These percentages were respectively 80% and 64% (p=0.2) at 1 year, 77% and 38% (p=0.01) at 18 months and 77% and 22% (p<0.001) at 2 years.

Using Cox regression model and multivariate analysis, two factors were associated with an evolution without clinical failure: combination therapy (HR=6.29 (95% CI 2.98 to 13.26); p<0.001) and lack of pharmacokinetic failure during the follow-up prior to clinical failure defined using a drug-sensitive assay (HR=8.32 (95% CI 3.69 to 16.45); p<0.001). Conversely, type of disease or of anti-TNF before inclusion, previous immunosuppressor use, titres of antidrug antibodies to the first anti-TNF (≥ or <100 ng/mL) or C reactive protein (CRP) levels were not associated significantly with clinical failure (table 2).

Table 2 Factors associated with an evolution without clinical failure (Cox regression model)

Variables	P values	HR (95% CI)
Combination therapy	<0.001	6.29 (2.98 to 13.26)
Crohn's disease	0.16	1.22 (0.41 to 8.21)
Men	0.39	0.84 (0.37 t o4.83)
Age	0.57	1.02 (0.78 to 3.92)
Duration of the disease	0.84	1.29 (0.58 to 6.82)
CRP at inclusion	0.40	0.79 (0.38 to 5.17)
Titer of antidrug antibodies (≥100 ng/mL)	0.45	1.12 [0.74–4.44)
Previous anti-TNF	0.60	1.32 [0.58–4.78]
Previous immunosuppressors	0.53	0.74 [0.32–6.21)
Lack of pharmacokinetic failure during the follow-up prior to clinical failure	<0.001	8.32 [3.69–16.45)

CRP, C reactive protein; TNF, tumour necrosis factor.

Pharmacokinetic failure since randomisation during the follow-up

Using a drug-sensitive assay and definition of pharmacokinetic failure as undetectable trough levels of the drug (undetectable TLI or TLA) with high antibodies (ATI or ATA ≥20 ng/mL)

Development of unfavourable pharmacokinetics was significantly higher in the group monotherapy (log-rank test p<0.001 (HR=8.05 (95% CI 3.91 to 16.58)) (figure 5). The results were similar whatever the anti-TNF (IFX or ADA) used after the switch in monotherapy (ADA vs IFX; p=0.35) or in combination of treatment (ADA+AZA vs IFX+AZA; p=0.70) (figure 6). Combination of treatment decreased significantly the rates of pharmacokinetic failure compared with ADA monotherapy (ADA+AZA: HR=0.12 (95% CI 0.03 to 0.40); p<0.001 and IFX+AZA: HR=0.16 (95% CI 0.06 to 0.37); p<0.001). Evolution without pharmacokinetic failure differed significantly between the two groups of patients from 12 months (p=0.04) but was not significantly different at 6 months (p=0.12). At 6 months, the percentages of patients without pharmacokinetic

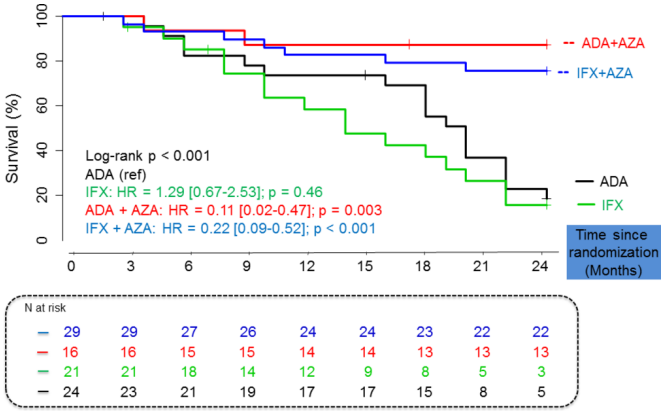

Figure 4 Evolution without clinical failure according to treatment. ADA, adalimumab; AZA, azathioprine; IFX, infliximab.

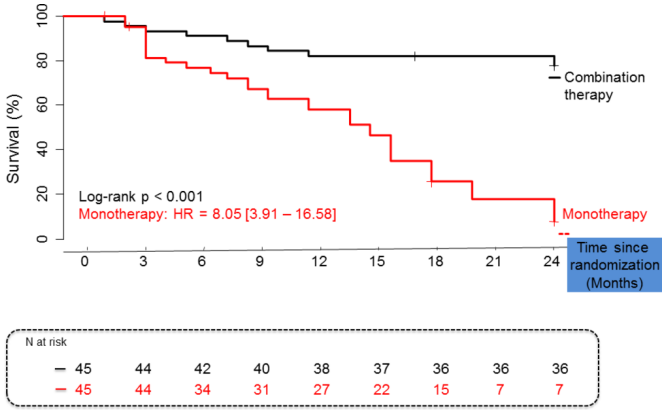

Figure 5 Evolution without unfavourable pharmacokinetics using a drug-sensitive assay.

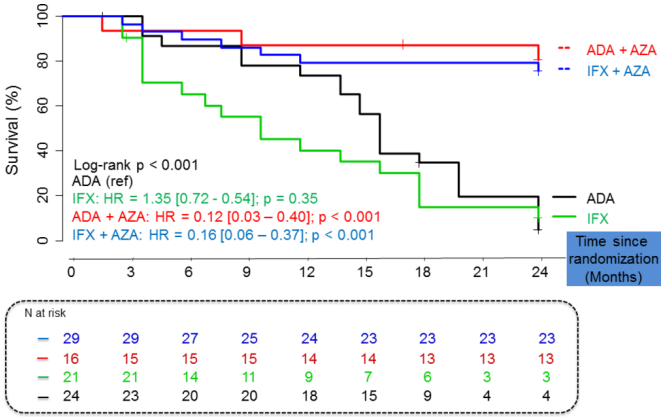

Figure 6 Evolution without unfavourable pharmacokinetics using a drug-sensitive assay according to treatment. ADA, adalimumab; AZA, azathioprine; IFX, infliximab.

failure were 90% and 75% under combination of treatment and monotherapy, respectively (p=0.7). These percentages were, respectively, 78% and 60% (p=0.25) at 1 year, 78% and 42% (p=0.02) at 18 months and 78% and 22% (p<0.001) at 2 years. At 18 and 24 months, 80% and 87% of patients who were in clinical failure had developed unfavourable pharmacokinetics of the second anti-TNF.

Using Cox regression model, only the factor combination of treatment was significantly associated with an evolution without pharmacokinetic failure (p<0.001) (table 3). Type of disease or of anti-TNF before inclusion, previous immunosuppressor use or CRP levels were not associated significantly with the evolution without pharmacokinetic failure.

A sensitivity analysis was performed in patients with very high (≥100 ng/mL) or lower titres (<100 ng/mL) of antidrug antibodies to the first anti-TNF at the inclusion. The immunogenic free survival after the inclusion was similar in patients with very high or lower titres of antibodies to the first anti-TNF (p=0.14; HR=1.52 (95% CI 0.86 to 2.67)) (online supplementary figure 1).

Using a drug-tolerant assay and definition of pharmacokinetic failure at the time when a positive antidrug antibody was isolated (>2 µg/mL eq)

Development of antibodies against anti-TNF drug using a drug-tolerant assay was significantly higher in the group monotherapy (log-rank test p<0.001 (HR=3.37 (95% CI 1.94 to 5.86)) (figure 7). The results were similar whatever the anti-TNF (IFX or ADA) used after the switch in monotherapy (ADA vs IFX; p=0.77) and in combination of treatment ADA+AZA (HR=0.61 (95% CI 0.30 to 1.24); p=0.17) (figure 8). Combination of treatment IFX+AZA decreased significantly the rates of pharmacokinetic failure compared with ADA monotherapy (HR=0.18 (95% CI 0.08 to 0.41); p<0.001) (figure 8). Evolution without pharmacokinetic failure was significantly different between the four groups of patients from 15 months (p=0.04) but was not significantly different at 6 months and 12 months (p=0.32 and p=0.12). Finally, 9% of patients developed transient antibodies.

Detection of antibodies against anti-TNF drug at 6 months using a drug-tolerant assay predicted an immunogenic failure defined with a drug-sensitive assay (no detectable drug and high antibodies) with a sensitivity of 57.1%, a specificity of 92.7% and positive and negative predictive values of 90.3% and 64.4%. The online supplementary figure 2 reported time to immunogenic failure using a drug-sensitive assay according to the detection of antibodies using a drug-tolerant assay at 6 months. Contrary to the association between clinical failure and immunogenic failure using a drug-sensitive assay, double-positive status ((detectable drug with positive antibodies) found by combining the results of the two assays) was not significantly associated with clinical failure (HR=1.3 (95% CI 0.3 to 6.8); p=0.51). In other words, all patients except one (under ADA monotherapy) with undetectable drug and antibodies measured whatever the two assays experienced clinical failure.

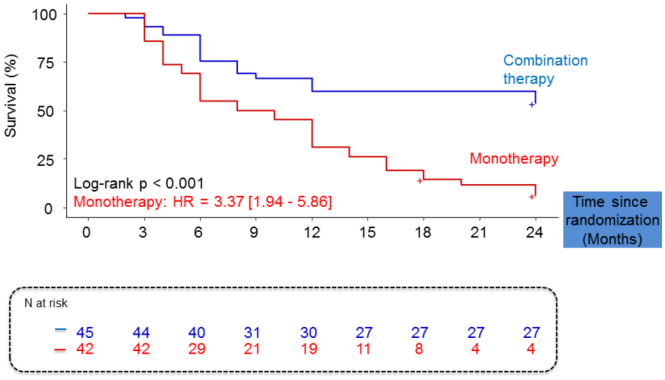

Figure 7 Evolution without unfavourable pharmacokinetics using a drug-tolerant assay.

Table 3 Factors associated with an evolution without pharmacokinetic failure (Cox regression model)

Variables	P values	HR (95% CI)
Combination therapy	<0.001	8.05 (3.91 to 16.58)
Crohn's disease	0.22	1.09 (0.71 to 2.24)
Men	0.79	0.95 (0.68 to 1.99)
Age	0.36	1.29 (0.58 to 1.78)
Duration of the disease	0.91	1.38 (0.92 to 2.39)
CRP	0.65	1.46 (0.68 to 3.96)
Previous anti-TNF	0.70	0.72 (0.54 to 4.58)
Previous immunosuppressors	0.55	1.03 (0.71 t o1.48)
Titer of antidrug antibodies (≥100 ng/mL)	0.14	0.62 (0.41 to 1.55)

CRP, C reactive protein; TNF, tumour necrosis factor.

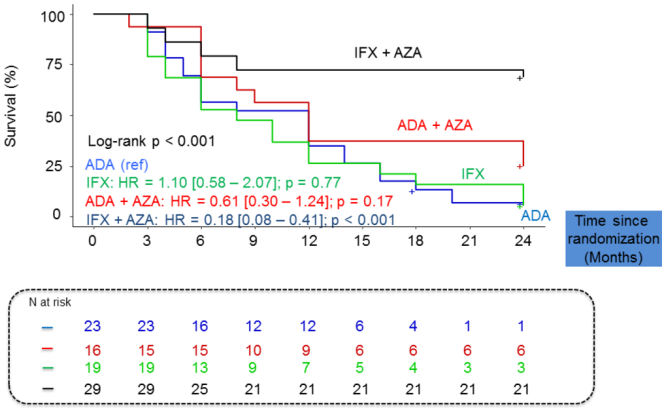

Figure 8 Evolution without unfavourable pharmacokinetics using a drug-tolerant assay according to treatment. ADA, adalimumab; AZA, azathioprine; IFX, infliximab.

Adverse events since randomisation

Forty-three side effects were recorded during the follow-up (20 vs 23 between mono and combination of treatment; p=0.8) (online supplementary table 1). In the two groups of patients, two patients reported serious adverse events requiring stopping treatment: one acute sarcoidosis and one case of breast cancer under monotherapy, one unexplained severe fever and one thymoma in a young men of 30 years old under the association anti-TNF with AZA (online supplementary table 1).

DISCUSSION

In this randomised study aiming to compare two strategies for immune-mediated loss of response to an optimised anti-TNF agent, we showed that the clinical and pharmacokinetic evolution was significantly more favourable after a switch to a second anti-TNF with AZA compared with a switch without AZA (≈80% vs 20% at 2 years). Only the use of combination therapy was significantly associated with favourable outcomes after anti-TNF switch.

Our remission rates after a switch in monotherapy (20% at 2 years) may be at first glance contradictory to other cohort data reporting significantly more favourable remission rates after change to another anti-TNF agent alone. In a retrospective work, Afif *et al*[15] reported clinical response rates after switching in nearly 92% of IBD patients with an immune-mediated pharmacokinetic failure with a median time of follow-up of 50 weeks. Likewise, in a prospective study of patients with IBD on ADA therapy, we showed that the switch to IFX without immunosuppressive drug after ADA optimisation in immunogenic-failed patients was associated with clinical remission rates of nearly 80% at 1 year.[16] However, no studies have reported data with longer longitudinal monitoring and have analysed the pharmacokinetic evolution of the second anti-TNF. In a recent prospective work, we have shown that the pharmacokinetic profiles of the first anti-TNF in case of loss of response have an impact on the pharmacokinetics of the second anti-TNF and on the clinical response at 2 years.[6] Eighty-five per cent of IBD patients with an immunogenic failure developed antidrug antibodies after a change to another anti-TNF agent alone, and they were associated with therapeutic failure at 2 years. In the present randomised study, we showed that at 1 year 64% of switched patients to monotherapy were in clinical remission and 60% of patients had not developed unfavourable pharmacokinetics, a feature that could eventually be superimposed on switch studies to another anti-TNF alone. In addition, the time to loss of response due to immunogenic failure was longer (beyond 15 months). Thus, although our study does not allow to analyse the optimal duration of combination therapy after switching, the late appearance of an immunogenic failure in the case of monotherapy pleads for a duration of combination therapy of at least 2 years. Moreover, the development of an immune-mediated pharmacokinetic failure occurred later after a second line of anti-TNF than it occurred after a first line of anti-TNF since 90% of patients who developed ATI under a first line of IFX did so within 12 months of therapy, as reported by Ungar *et al*.[7]

We also showed that combination therapy was significantly more effective in decreasing clinical failure than a switch in monotherapy regardless of the anti-TNF used. While most studies have shown the value of the addition of an immunosuppressant drug in reducing the risk of immunogenicity with IFX,[8 17] the data are more conflicting with ADA. In the study of Ungar *et al*,[18] the authors did not show that combination therapy significantly decreased the appearance of antibodies to ADA (ATA), in contrary to the PANTS study[8] that showed a significant protective effect of immunosuppressant drugs against the appearance of ATA. When we analysed immunogenic failure with a drug-sensitive assay, combination of therapy was more effective to decrease this risk with IFX and ADA. However, when we used a drug-tolerant assay previously used in the study of Ungar *et al*, the association IFX+AZA was significantly more effective than the association ADA+AZA, which was only numerically better than monotherapy. Therefore, the discordant results could be related at least in part to the assays used to monitor antidrug antibodies (same drug-tolerant assay in the case of Ungar *et al*[18] and of the present study vs another drug-tolerant assay in the PANTS study[8]). In our study combining the results of the two assays (drug sensitive and drug tolerant), 31 out of 90 patients were found to develop antidrug antibodies in the presence of anti-TNF (ie, 'double-positive status') at 6 months and 26 of them (84%) became 'antidrug antibody positive/drug negative' in subsequent sera measurements. These results are significantly higher than those reported by Ungar *et al*[16] with only 33% of patients who became ATA positive/drug negative, but the median of follow-up was significantly shorter (44 weeks) than in our study and they only analysed ADA treatment. Moreover, in contrary to the association between clinical failure and immunogenic failure defined by using a drug-sensitive assay in our study, double-positive status was not significantly associated with clinical failure (HR=1.3 (95% CI 0.3 to 6.8); p=0.51). These results are in accordance with those from Ungar *et al*[18] and from the post hoc analysis of the Trough Concentration Adapted Infliximab Treatment (TAXIT) trial.[19] However, we showed that these antibodies isolated using a drug-tolerant assay predicted an immunogenic failure defined with a drug-sensitive assay (no detectable drug and high antibodies) with a high positive predictive value (90.3%).

We used full doses of AZA and did not assess the levels of 6-thioguanine nucleotides (6-TGNs). We cannot therefore conclude whether lower doses could be sufficient to avoid an unfavourable evolution after switching. In a previous work, we have shown that half doses of AZA were able to prevent the development of unfavourable pharmacokinetics under IFX.[20] Likewise, Yarur *et al*[21] have shown that levels of 6-TGN <125 pmoles/10^8 cells were sufficient to prevent the immunogenicity-based failure under IFX. For ADA, no studies showed that lower levels of 6-TGN reduced the immunogenic risk. In a recent study, thiopurines dosed to a therapeutic 6-TGN level in combination with ADA are more effective than subtherapeutic thiopurine-based combination therapy or ADA monotherapy during induction and maintenance in patients with long-standing CD.[22] Similarly, we cannot know whether the use of methotrexate would have led to the same result in this indication. However, in patients with immune-mediated loss of response, the addition of AZA or methotrexate allowed to reverse this immunogenic failure.[9 23]

Whether the results would have been the same in patients with immune-mediated failure but without therapeutic optimisation remain to be determined as dose optimisation only provides sustainable response rates in less than 20% of cases.[16 24] The addition of immunosuppressive drugs can decrease antidrug antibody levels and increase trough levels of anti-TNF, but the time required to achieve clinical improvement is long (almost 6 months).[10] Our patients suffered from active disease at the inclusion, which precluded to propose them only addition of immunosuppressive drugs.

Our study has some limitations. It was an open study comparing two therapeutic strategies without using placebo in anti-TNF monotherapy arms. However, we used objective markers to define

clinical failure as faecal calprotectin in CD and endoscopy in UC. In addition, after randomisation, pharmacokinetic measurements were performed blindly without knowledge of clinical data and were returned only at the end of the study. We cannot assess the effect of optimisation of the second anti-TNF in case of therapeutic failure since the study was not designed to analyse specifically this point. Our study did not report any primary non-response after randomisation in either monotherapy or combination therapy. These favourable results do not agree with many studies showing primary non-response ranging from 20% to 30% after anti-TNF switch.[8 18 24] However, in our study, patients who had presented a non-response to a first anti-TNF were not included. Our study randomised only 90 patients, which was sufficient and confirmed our statistical hypothesis. In addition, we chose in the statistical analysis the worst hypothesis to compare the two strategies confirming the superiority of combination therapy despite this disadvantageous hypothesis.

In case of loss of response under anti-TNF with an optimised dose and with undetectable levels of anti-TNF and high antidrug antibodies, the switch to another anti-TNF in combination with AZA is significantly better than a switch without AZA in terms of clinical and pharmacokinetic evolution and with comparable tolerance. In case of immune-mediated loss of response to a first anti-TNF, AZA should be used in combination with the second anti-TNF.

Author affiliations
[1]Department of Gastroenterology, CHU Saint-Étienne, Saint-Etienne, Rhône-Alpes, France
[2]Department of Gastroenterology, Hospital Lyon-South, Pierre-Benite, France
[3]Department of Immunology, CIC 1408, GIMAP, CHU Saint-Étienne, Saint-Etienne, Rhône-Alpes, France
[4]Gastro-enterology Department, Centre Hospitalier du Forez à Montbrison Instituts de Formation, Montbrison, Auvergne-Rhône-Alpes, France
[5]Department of Statistics, UCBL, Villeurbanne, Auvergne-Rhône-Alpes, France
[6]Department of gastroenterology, University Hospital Centre Nancy, Nancy, Lorraine, France
[7]INSERM U954, Nancy 1 University, Nancy, Lorraine, France

Contributors XR, SP, GD, EDT, PV, A-EB, GB, SN and BF performed the research; XR, SP, LP-B, EDT and BF designed the research study. SP, GD and NW analysed the data. XR, GB, SN, LP-B, J-MP, SP and BF wrote the paper. All authors approved the final version of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests XR was speaker for MSD, Abbvie and Takeda and participated in an advisory board member for MSD, Takeda, Janssen and Pfizer. SP was speaker for Theradiag and MSD. EDT was speaker for Ferring, MSD and Abbvie. GB, Berger, Phelip, Vedrines and Duru. LP-B received Honoraria from Merck, Abbvie, Janssen, Genentech, Mitsubishi, Ferring, Norgine, Tillots, Vifor, Hospira/Pfizer, Celltrion, Takeda, Biogaran, Boerhinger-Ingelheim, Lilly, HAC-Pharma, Index Pharmaceuticals, Amgen, Sandoz, Forward Pharma GmbH, Celgene, Biogen, Lycera and Samsung Bioepis. SN was speaker for MSD, Hospira and HAC Pharma and participated in an advisory board member for Hospira. BF was speaker for Hospira, Abbvie and MSD.

Patient consent for publication Not required.

Ethics approval This study was approved by the Ethics Committee Board of Montbrison Hospital and the Centre National Informatique et Liberté (number: 1849323).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iDs
Laurent Peyrin-Biroulet http://orcid.org/0000-0003-2536-6618
Stephane Paul http://orcid.org/0000-0002-8830-4273

REFERENCES

1 Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009;104:760–7.
2 Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of response and need for adalimumab dose intensification in Crohn's disease: a systematic review. *Am J Gastroenterol* 2011;106:674–84.
3 Casanova MJ, Chaparro M, Mínguez M, *et al*. Effectiveness and safety of the sequential use of a second and third anti-TNF agent in patients with inflammatory bowel disease: results from the Eneida registry. *Inflamm Bowel Dis* 2019;104.
4 Vande Casteele N, Khanna R, Levesque BG, *et al*. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. *Gut* 2015;64:1539–45.
5 Mitrev N, Vande Casteele N, Seow CH, *et al*. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther* 2017;46:1037–53.
6 Roblin X, Verot C, Paul S, *et al*. Is the pharmacokinetic profile of a first anti-TNF predictive of the clinical outcome and pharmacokinetics of a second anti-TNF? *Inflamm Bowel Dis* 2018.
7 Ungar B, Chowers Y, Yavzori M, *et al*. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014;63:1258–64.
8 Kennedy NA, Heap GA, Green HD, *et al*. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *Lancet Gastroenterol Hepatol* 2019;4:341–53.
9 Ben-Horin S, Waterman M, Kopylov U, *et al*. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2013;11:444–7.
10 Ungar B, Kopylov U, Engel T, *et al*. Addition of an immunomodulator can reverse antibody formation and loss of response in patients treated with adalimumab. *Aliment Pharmacol Ther* 2017;45:276–82.
11 Van Assche G, Magdelaine–Beuzelin C, D'Haens G, *et al*. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. *Gastroenterology* 2008;134:1861–8.
12 Ben-Horin S, Yavzori M, Katz L, *et al*. The immunogenic part of infliximab is the F(ab')2, but measuring antibodies to the intact infliximab molecule is more clinically useful. *Gut* 2011;60:41–8.
13 Mallinckrodt CH, Bell J, Liu G, *et al*. Aligning estimators with estimands in clinical trials: putting the ICH E9(R1) Guidelines Into Practice. *Ther Innov Regul Sci* 2019;2168479019836979.
14 Ratitch B, O'Kelly M, Tosiello R. Missing data in clinical trials: from clinical assumptions to statistical analysis using pattern mixture models. *Pharm Stat* 2013;12:337–47.
15 Afif W, Loftus EV, Faubion WA, *et al*. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010;105:1133–9.
16 Roblin X, Rinaudo M, Del Tedesco E, *et al*. Development of an algorithm incorporating pharmacokinetics of adalimumab in inflammatory bowel diseases. *Am J Gastroenterol* 2014;109:1250–6.
17 Colombel JF, Sandborn WJ, Reinisch W, *et al*. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010;362:1383–95.
18 Ungar B, Engel T, Yablecovitch D, *et al*. Prospective Observational Evaluation of Time-Dependency of Adalimumab Immunogenicity and Drug Concentrations: The POETIC Study. *Am J Gastroenterol* 2018;113:890–8.
19 Van Stappen T, Vande Casteele N, Van Assche G, *et al*. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. *Gut* 2018;67:818-826.
20 Roblin X, Boschetti G, Williet N, *et al*. Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. *Aliment Pharmacol Ther* 2017;46:142–9.
21 Yarur AJ, Kubiliun MJ, Czul F, *et al*. Concentrations of 6-thioguanine nucleotide correlate with Trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. *Clin Gastroenterol Hepatol* 2015;13:1118–24.
22 Kariyawasam VC, Ward MG, Blaker PA, *et al*. Thiopurines dosed to a therapeutic 6-thioguanine level in combination with adalimumab are more effective than subtherapeutic Thiopurine-based combination therapy or adalimumab monotherapy during induction and maintenance in patients with long-standing Crohn's disease. *Inflamm Bowel Dis* 2017;23:1555–65.
23 Strik AS, van den Brink GR, Ponsioen C, *et al*. Suppression of anti-drug antibodies to infliximab or adalimumab with the addition of an immunomodulator in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2017;45:1128–34.
24 Vande Casteele N, Gils A, Singh S, *et al*. Antibody response to infliximab and its impact on pharmacokinetics can be transient. *Am J Gastroenterol* 2013;108:962–71.