SUPPLEMENTARY TEXT

PROTOCOL AMENDMENTS

The protocol was amended seven times. Key changes included: permitting enrolment of patients who had failed or demonstrated intolerance to either immunosuppressant therapies *or* anti-tumour necrosis factor (TNF) agents, allowing enrolment based on a colonoscopy performed up to 8 weeks before study entry, adding cardiac monitoring, clarifying entry criteria and the analysis plan and eliminating some exploratory testing.

METHODS

Patients

For anti-TNF agents, treatment failure was defined as either inability to respond to initial therapy or relapse following the original response to therapy; intolerance was defined as having experienced clinically significant side effects, including hypersensitivity.

For immunosuppressant therapies, treatment failure was defined as continued disease activity despite treatment with a therapeutic dose of azathioprine, 6-mercaptopurine and/or methotrexate; intolerance was defined as a history of having experienced an unacceptable or dose-limiting toxicity associated with the use of the agent.

Efficacy evaluations and statistical analysis

Detail of flow cytometry method

Briefly, aliquots (100 µL) of sodium heparin anti-coagulated blood were incubated with 30 µL of the antibody cocktail (reagents purchased from Becton Dickinson, San Jose, CA, USA): CD45RO-FITC Cat#555492, β7-integrin or rat immunoglobulin (Ig)G2a isotype control-PE Cat#555945 and 555844, respectively, CD4-PerCPCy5.5 Cat#332772, CD27-APC Cat#337169 and CD3-APC-H7 Cat#641396 in BD TruCount tubes (Cat#340334) at room temperature for 30 min. To each tube, 1 mL of 1X BD PharmLyse solution (Cat# 555899) was added and shaken by hand and incubated for 30 min at room temperature whilst being protected from light for 30 min. Lysed blood was then acquired by the FACSCanto™ II (BD Biosciences, San Jose, CA, USA) within 2 hours of preparation. β_7+ data were reported as percent of overall CD4+ expressing cells, absolute number (cells/µL) and molecules of equivalent soluble fluorochrome (MESF), the unit measure of β_7+ protein expression on central memory T cells.

RESULTS

Patient disposition and baseline characteristics

Anti-TNF washout

Fifty six patients discontinued anti-TNF therapy <12 weeks before randomization (Supplementary Table S2): 14, 12, 12, and 18 patients in the placebo and PF-00547659 22.5-mg, 75-mg and 225-mg groups, respectively. Two of the 56 patients did not undergo colonoscopy.

Table S2 Timing of anti-TNF therapy discontinuation before randomization

Anti-TNF washout period (wk)	n (%)
<6	0
6 to <8	20 (35.7)
8 to <10	16 (28.6)
10 to <12	20 (35.7)

Interval between Anti-TNF discontinuation and colonoscopy

Ten patients (3, 5, and 2 in the placebo and PF-00547659 75-mg and 225-mg groups, respectively) had colonoscopy >4 weeks before randomization and anti-TNF discontinuation <12 weeks before randomization. Five patients (2, 2, and 1 in the placebo, 75-mg, and 225-mg groups) had colonoscopy before discontinuation of anti-TNF therapy.

Biomarkers, pharmacokinetics and anti-drug antibody formation

No apparent correlation was observed between late discontinuation of anti-TNF therapy and findings related to biomarkers, pharmacokinetics, or anti-drug antibodies in this study.

Details of ADA analysis

Of the 30 anti-drug antibodies (ADA) samples that were confirmed positive, eight were from patients treated with PF-00547659 22.5 mg, 10 from patients receiving 75 mg and two from patients treated with 225 mg. Of the eight patients with confirmed ADA at baseline, three were also positive post-baseline (one subject in each of the 22.5-, 75- and 225-mg groups), with no indication of treatment-enhanced ADA response (i.e. greater ADA titre post-treatment as compared with baseline).