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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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TOPPIC Web appendix

Contents

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Supplementary table 1: Inclusion and Exclusion Criteria for the TOPPIC Trial

Inclusion Criteria

A patient will be considered eligible for inclusion in the study if all of the following criteria apply:

1. At least 16 years of age in Scotland and 18 years of age in England and Wales.
2. Established diagnosis of Crohn's disease confirmed at recent resection.
3. Ileocolonic or small bowel resection within 3 months before screening.
4. No more than 100 cm of fixed small bowel resected in total. Previous ileocolonic resection is acceptable.
5. Able to start oral nutrition within the first 2 postoperative weeks.
6. Normal or heterozygous TPMT (activity present or reduced consistent with carrier status).
7. Able to provide written informed consent prior to screening and to comply with the requirements of the study protocol.
8. Off antibiotics 2 weeks prior to randomisation.

Exclusion Criteria

A patient will <u>not</u> be eligible for inclusion in the study if any of the following criteria apply:

1. Pregnancy at baseline or breast feeding.
2. A known hypersensitivity or intolerance to 6MP
3. Pancreatitis associated with azathioprine.
4. Receiving an experimental treatment for Crohn's disease in the 4 weeks prior to study entry.
5. Known to require further surgery at study entry i.e. for the removal of an abscess developing from the primary surgery.
6. Strictureplasty procedure alone
7. (Please note that strictureplasty and resection procedure together will not be considered an exclusion.)
8. Presence of stoma.
9. Significant haematological, renal or hepatic dysfunction or clinically important lung disease (i.e. liver function tests (except GGT) >x2 upper limit of normal, Haemoglobin ≤10, total white blood cell count <3.5, Neutrophils <1.5, Platelets <100x10^9/l).
10. Systemic infection including hepatitis B, hepatitis C, HIV and active TB.
11. A diagnosis of indeterminate colitis or ulcerative colitis.
12. A history of illicit drug or alcohol abuse in the 1 year prior to study entry.
13. Active or untreated malignancy (excluding basal cell carcinoma and insitu tumours). (Patients who have had successful treatment for malignancy and have been in remission for more than 5 years may be considered for inclusion only after detailed discussion with, and written approval, from the patient's medical oncologist.)
14. Presence of a medical or psychiatric condition, disease or laboratory abnormality that in the opinion of the PI may place the subject at unacceptable risk during the study.
15. Homozygous deficient for TPMT (absent activity)
16. Evidence of untreated post-operative infection e.g. clostridium difficile, urinary tract infection or chest infection. If these have been appropriately treated in the opinion of the PI, and inclusion criteria 8 is met, this will not be considered an exclusion.
17. Taking any medication for Crohn's disease.

Supplementary table 2: Dose reduction algorithm

Option 1	No action required as not deemed to be a safety issue (Some abnormal results will be at the discretion of the Investigators as to whether they require any action.)
Option 2	Hb<10.0: E-mail local nurse to telephone patient and arrange for review and assessment by Investigator as would be done in routine clinical practice. Anaemia could be caused by a number of reasons so this should not affect the study blind.
Option 3	Total White Cell Count 2.5-3.5, **or** Neutrophil count between 1.0 and 2.0 **or** any LFT (except GGT) between >2 and 5 times the upper limit of normal **or** Platelet count between 50-100x10^6/l: Email local nurse who will telephone patient to reduce dose. Ensure safety bloods are done within 1 week at local site. This should not affect the study blind.
Option 4	Total White Cell Count <2.5 or Neutrophil count <1.0 or Any LFT >x5 upper limit of normal or platelet count <50x106/l: Email and telephone local nurse who will telephone patient to discontinue the study drug, arrange for immediate patient review by Investigator and repeat bloods, at weekly intervals, until resolved. At the duty clinicians' discretion drug could be recommenced or the dose reduced [see Para 7.7 of Protocol Dose Reduction Schedule (Table 2)], if out of range values improve above the lower limits designated. Patient will continue as per protocol. Otherwise study drug would be stopped and patient should continue with scheduled visits but not take any more IMP. (The breaking of the study blind will only be performed where knowledge of the treatment is absolutely necessary for further management of the patient).

Supplementary table 3: Overview of Trial Procedures

Visit number	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Study Week No	**-4**	**-1**	**0**	**6**	**13**	**31**	**49**	**67**	**85**	**103**	**121**	**139**	**157**
Visit purpose	**Inpatient pre-assessment**	**Safety Visit**	**Randomisation and drug prescription**	**Interim safety assessment**	**Study visit**	**Study visit**	**Study visit**	**Study visit**	**Study visit**	**Study visit**	**Study visit**	**Study visit**	**Final study visit**
Inc/Exc Criteria	x												
Consent Form	x		x										
Patient Information Leaflet	x												
TPMT	x												
Stool Sample	x												
Pregnancy Test			x										
Clinic Visit			x	x	x	x	x	x	x	x	x	x	x
Medical History			x										
CDAI (Diary to be completed by patient 7 days prior to this visit)			x	x	x	x	x	x	x	x	x	x	x
Weight			x	x	x	x	x	x	x	x	x	x	x
Height			x										
Study Drug			x	x	x	x	x	x	x	x	x	x	
Physical exam inc vital signs			x	x	x	x	x	x	x	x	x	x	x
AEs		x	x	x	x	x	x	x	x	x	x	x	x
Blood Safety Monitoring*		x		x	x	x	x	x	x	x	x	x	x
6MP Metabolite Levels			x		x		x			x			x
Faecal Calprotectin			x		x		x			x			x
Colonoscopy & Biopsy							x						x
IBDQ, SF-36 & EQ-5D			x		x		x			x			x
Prohibited Meds				x	x	x	x	x	x	x	x	x	x
Genetic Studies			X										
Serological Studies			X										

Supplementary table 4: Recruitment to TOPPIC per centre

Centre	Principal Investigator	Number of patients	% of patients
Western General Hospital, Edinburgh	Dr Ian Arnott	78	32.5
Ninewells, Dundee	Dr Craig Mowat	21	8.8
Stobhill Hospital, Glasgow	Dr Aidan Cahill	19	7.9
Aberdeen Royal Infirmary, Abderdeen	Dr Malcolm Smith	17	7.1
Royal Devon and Exeter Hospital, Exeter	Dr Tariq Ahmad	11	4.6
John Radcliffe Hospital, Oxford	Dr Simon Travis	9	3.8
Glasgow Royal Infirmary, Glasgow	Dr John Morris	8	3.3
Royal Liverpool University Hospital, Liverpool	Sreedhar Subramanian	8	3.3
Leeds General Infirmary, Leeds	Dr John Hamlin	8	3.3
University Hospital Coventry, Coventry	Professor Chuka Nwokolo	6	2.5
Torbay Hospital, Torquay	Dr Cathryn Edwards	6	2.5
University Hospital of North Durham, Durham	Dr Anjan Dhar	6	2.5
Bristol Royal Infirmary, Bristol	Dr Tom Creed	5	2.1
UCLH, London	Dr Stuart Bloom	5	2.1
Singleton Hospital, Swansea	Dr Linzi Thomas	4	1.7
Rotherham General Hospital, Rotherham	Dr Mohamed Yousif	4	1.7
Manchester Royal Infirmary, Manchester	Dr Simon Campbell	3	1.2
Derriford Hospital, Plymouth	Dr Stephen Lewis	3	1.2
Hull Royal Infirmary, Hull	Dr Shaji Sebastian	3	1.2
Salford Royal Infirmary, Salford	Dr Simon Lal	2	0.8
Royal Free, London	Dr Charles Murray	2	0.8
Southampton General Hospital, Southampton	Dr Fraser Cummings	2	0.8
Queen's Medical Centre, Nottingham	Professor Chris Hawkey	2	0.8
Queen Elizabeth Hospital, Birmingham	Dr Jason Goh	2	0.8
Darlington Memorial Hospital, Darlington	Dr Anjan Dhar	2	0.8
Barts and the London, London	Dr James Lindsay	1	0.4
North Staffs, Stoke-on-Trent (now Royal Stoke University Hospital)	Dr Sandip Sen	1	0.4
St Marks, London	Dr Naila Arebi	1	0.4
Raigmore, Inverness	Dr Lindsay Potts	1	0.4

Supplementary table 5: Data and sample completion rates across TOPPIC trial outcomes

Parameter	Timepoint post-randomisation	6MP (n=128)	Placebo (n=112)	Overall (n=240)
Clinical data	**Week 0**	128 (100)	112 (100)	240 (100)
Clinical data	**Week 6**	123 (96.1)	108 (96.4)	231 (96.3)
Clinical data	**Week 13**	117 (91.4)	107 (95.5)	224 (93.3)
Clinical data	**Week 31**	114 (89.1)	99 (88.4)	213 (88.8)
Clinical data	**Week 49**	111 (86.7)	97 (86.6)	208 (86.7)
Clinical data	**Week 67**	105 (82.0)	87 (77.7)	192 (80.0)
Clinical data	**Week 85**	100 (78.1)	83 (74.1)	183 (76.3)
Clinical data	**Week 103**	98 (76.6)	78 (69.6)	176 (73.3)
Clinical data	**Week 121**	94 (73.4)	74 (66.1)	168 (70.0)
Clinical data	**Week 139**	89 (69.5)	71 (63.4)	160 (66.7)
Clinical data	**Week 147**	89 (69.5)	71 (63.4)	161 (67.1)
Colonoscopy	**Week 49**	95 (74)	77 (69)	172 (72)
Colonoscopy	**Week 157**	69 (54)	59 (53)	128 (53)
Calprotectin	**Week 0**	108	96	204
Calprotectin	**Week 13**	94	87	181
Calprotectin	**Week 49**	87	67	154
Calprotectin	**Week 103**	69	55	124
Calprotectin	**Week 157**	65	52	117
6-TGN	**Week 0**	124	110	234
6-TGN	**Week 13**	114	97	211
6-TGN	**Week 49**	102	86	188
6-TGN	**Week 103**	82	65	147
6-TGN	**Week 157**	64	57	121
IBDQ	**Week 0**	128	111	239
IBDQ	**Week 13**	117	106	223
IBDQ	**Week 49**	109	94	203
IBDQ	**Week 103**	97	75	172
IBDQ	**Week 157**	86	69	155

Supplementary table 6: Sensitivity and specificity (and 95% CIs) of faecal calprotectin for prediction of post-operative endoscopic recurrence of Crohn's disease (Rutgeerts' ≥ i2)

Endoscopic Parameter	FC cut-off (µg/g)	Sensitivity	Specificity	PPV	NPV
Recurrence (≥i2)	50	84.4% (77.0%, 91.9%)	44.4% (35.6%, 53.1%)	52.4% (44.3%, 60.5%)	79.7% (70.2%, 89.2%)
	100 (optimum*)	72.2% (63.0%, 81.5%)	62.1% (53.6%, 70.6%)	58.0% (48.9%, 67.2%)	75.5% (67.1%, 83.8%)
Remission (i0)	50	51.8% (38.7%, 64.9%)	74.7% (67.9%, 81.5%)	42.0% (30.4%, 53.7%)	81.4% (75.0%, 87.7%)
	100	67.9% (55.6%, 80.1%)	59.5% (51.8%, 67.2%)	37.3% (27.9%, 46.6%)	83.9% (77.1%, 90.7%)
	60 (optimum*)	55.4% (42.3%, 68.4%)	70.9% (63.8%, 78.0%)	40.3% (29.3%, 51.2%)	81.8% (75.3%, 88.2%)

These data derived from combined Visit 6 (week 49) and Visit 12 (week 157) data (n = 214)

* Optimum cut-off point calculated by maximising Youden's J statistic.

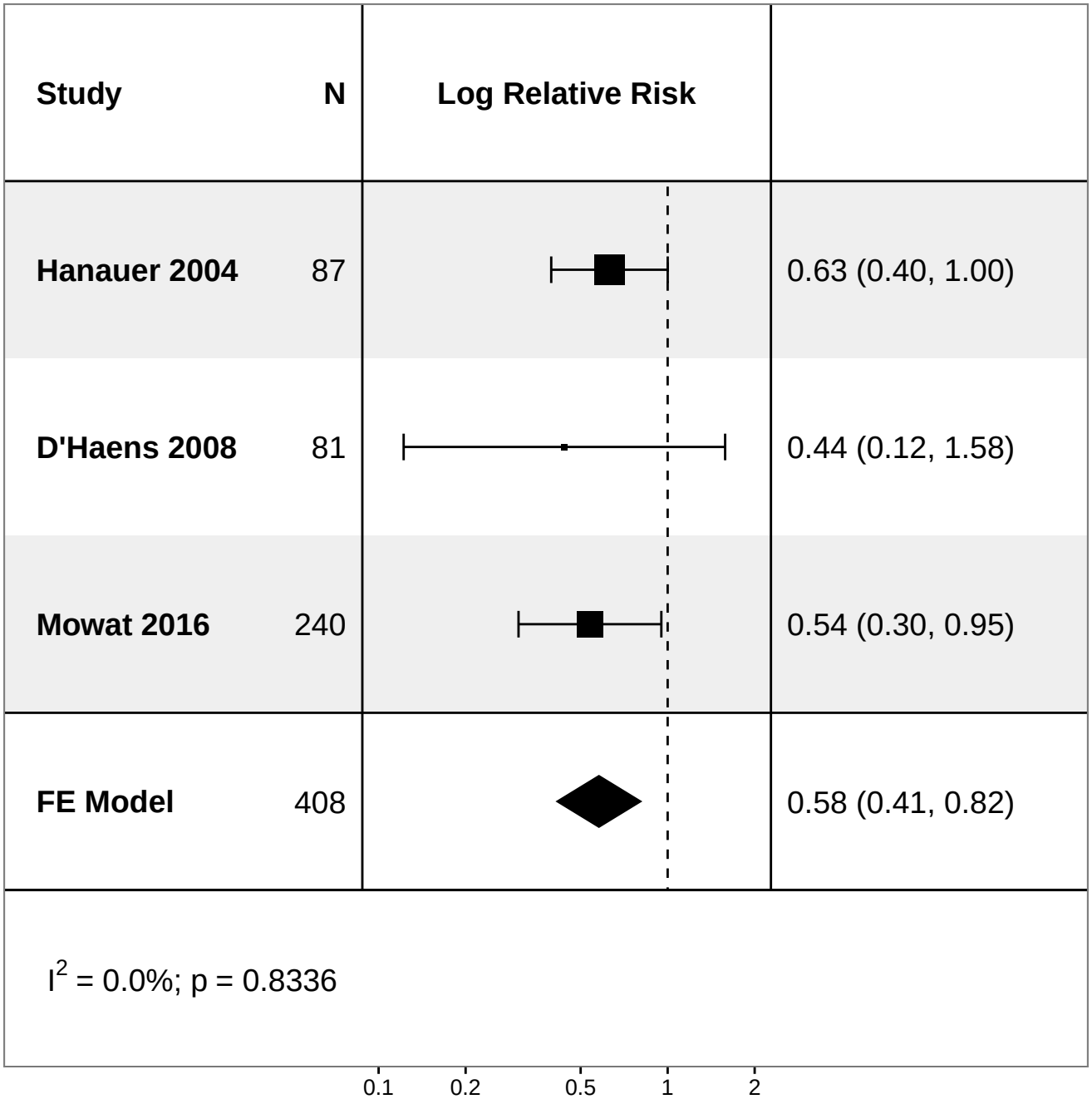
Supplementary table 7: Adverse events stratified by category, severity and treatment group

	Mercaptopurine				Placebo			
Category	**Mild**	**Moderate**	**Severe**	**Total**	**Mild**	**Moderate**	**Severe**	**Total**
Cancers	0	3	0	**3**	0	0	1	**1**
Deranged LFTs	0	4	0	**4**	0	5	0	**5**
GI Symptoms - Abdominal pain	41	64	26	**132**	32	85	24	**141**
GI Symptoms - Constipation/diarrhoea	23	24	7	**54**	17	31	8	**56**
GI Symptoms - Nausea/vomiting	25	42	11	**78**	18	21	2	**41**
GI Symptoms - Other	23	26	4	**53**	22	18	0	**40**
Headache	39	22	0	**61**	17	18	3	**38**
Infections	85	80	6	**171**	75	102	7	**184**
Joint pain/arthralgia	28	40	4	**72**	21	40	4	**65**
Pain	11	15	4	**30**	4	12	3	**19**
Pancreatitis	0	1	0	**1**	0	0	1	**1**
Rash	21	12	2	**35**	15	2	0	**17**
Worsening Crohn's	6	29	6	**41**	5	25	7	**37**
Other	97	106	9	**212**	70	72	11	**153**

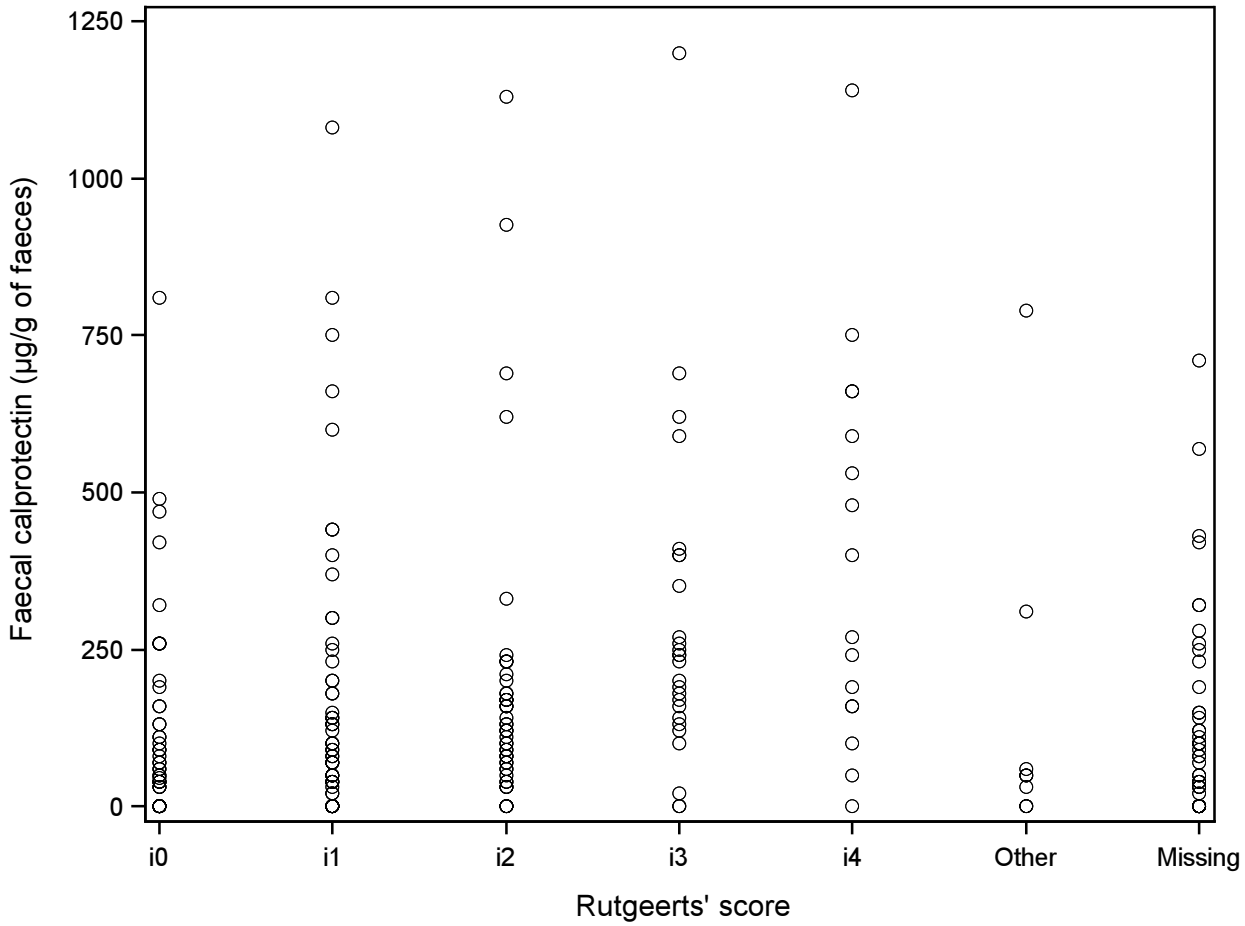
Numbers indicate total number of events. There was one case of abdominal pain in the mercaptopurine group with missing severity, and one mild event with a missing category.

Supplementary figure 1: Meta-analysis of published randomised controlled trial data comparing thiopurines to placebo for prevention of post-operative recurrence within 12 months in Crohn's disease

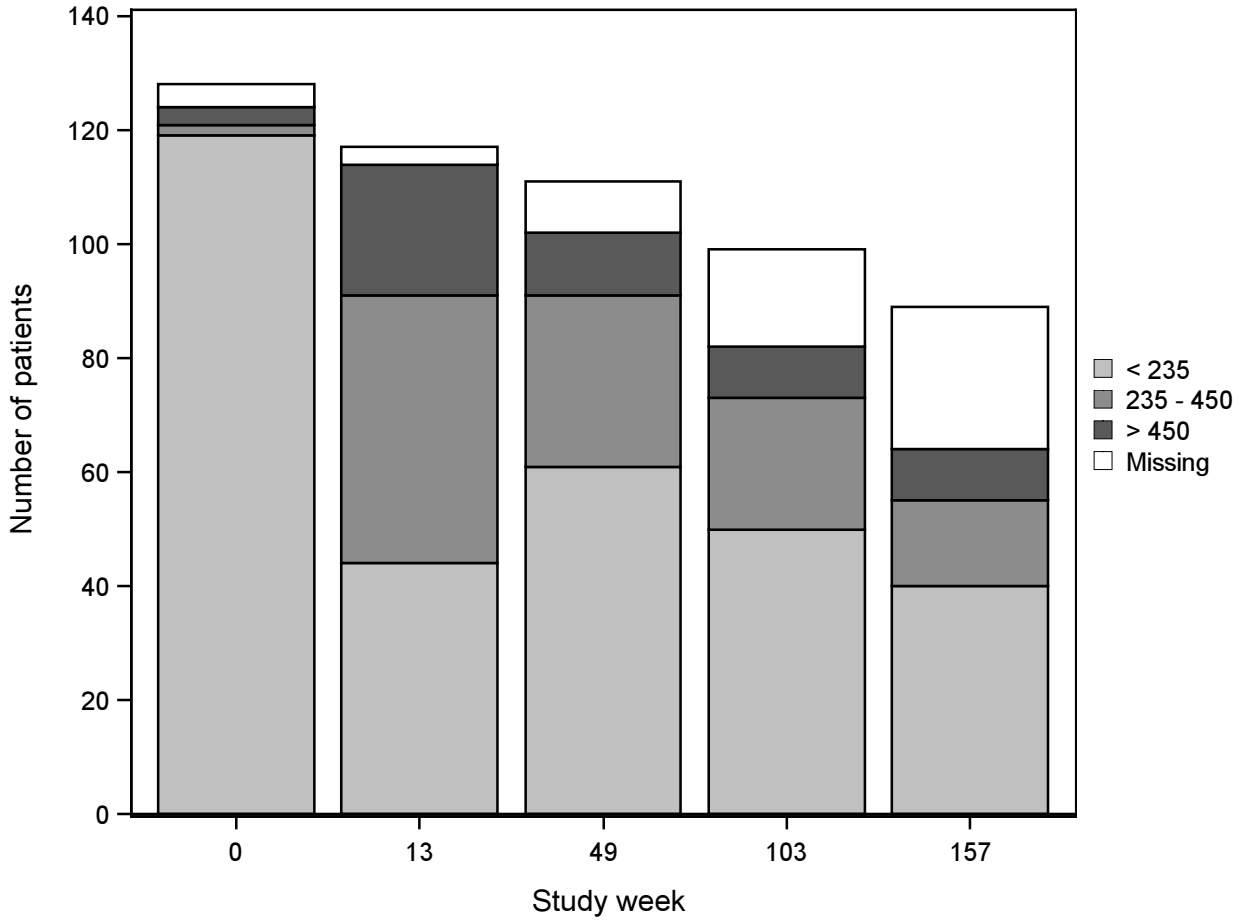
FE = Fixed effects



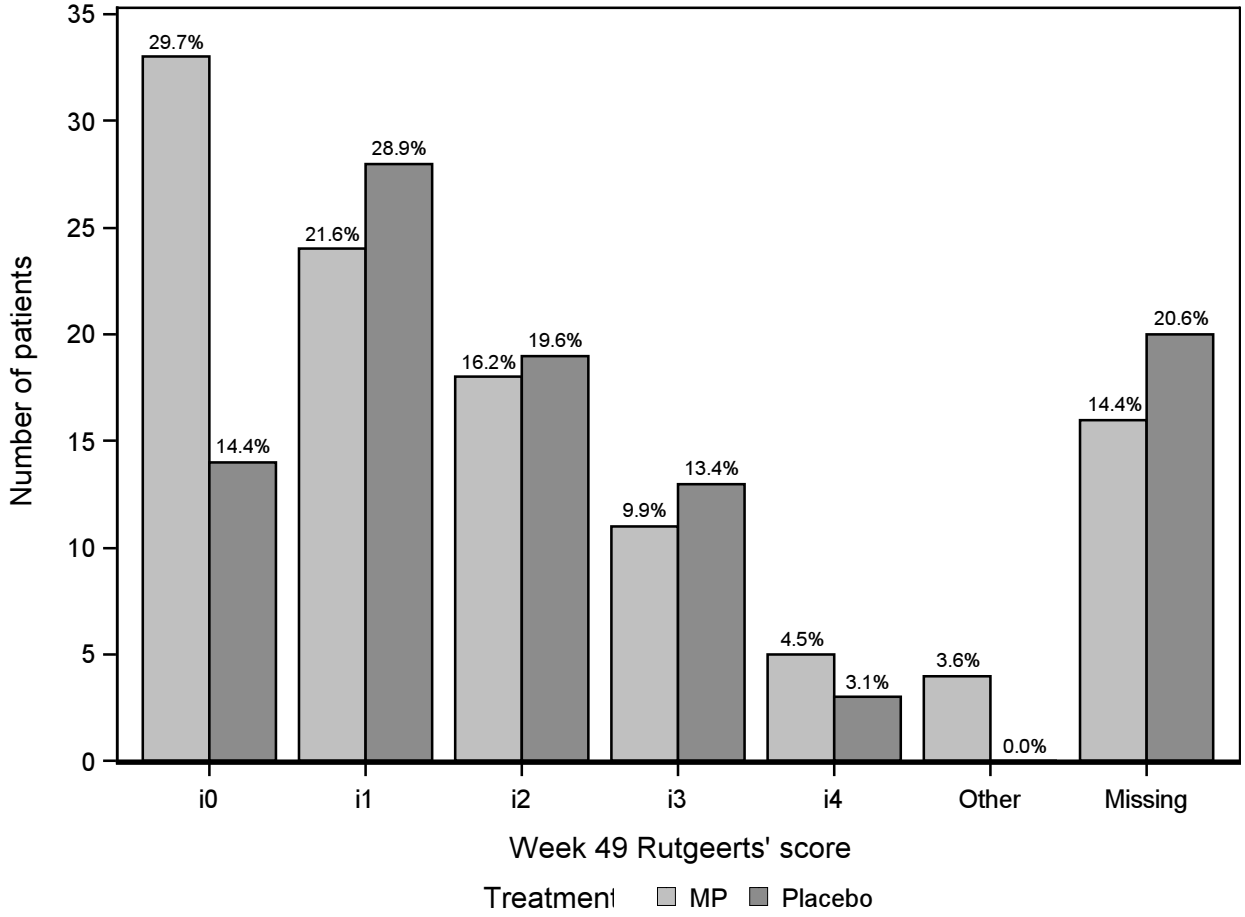
Supplementary figure 2: Faecal calprotectin concentrations measured at weeks 49 and 157 plotted against corresponding Rutgeerts' score for endoscopic recurrence of Crohn's disease



Supplementary figure 3: Thioguanine nucleotide (TGN) blood concentrations in patients receiving MP plotted by study week, and grouped according to therapeutic range (<235 – 450, pmol/8x108 RBC), n=128



Supplementary figure 4: Endoscopic recurrence of Crohn's disease as measured by Rutgeerts' score at Week 49 post-randomisation in patients on mercaptopurine (MP) vs placebo



Supplementary figure 5: Endoscopic recurrence of Crohn's disease as measured by Rutgeerts' score at Week 157 post-randomisation in patients on mercaptopurine (MP) vs placebo comparing MP vs placebo