Statistical Analysis Plan

TOPPIC
Randomised controlled trial of 6-mercaptopurine versus placebo to prevent recurrence of Crohn's disease following surgical resection

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Authors: Steff Lewis, Catriona Keerie

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Chief Investigator: Prof Jack Satsangi
j.satsangi@ed.ac.uk

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Signatures

Jack Satsangi / 1 Jun 15

Chief Investigator
Prof Jack Satsangi

Signature Date

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Trial Statistician
Dr Steff Lewis

Signature Date

1 Table of Contents

2 List of Abbreviations

6-MP	6-Mercaptopurine
CI	confidence interval
CONSORT	CONsolidated Standards of Reporting Trials
DMC	Data monitoring committee
ECTU	Edinburgh Clinical Trials Unit
EQ-5D	EuroQol 5 dimensions health outcome questionnaire
IBDQ	Inflammatory bowel disease questionnaire
IQR	Inter quartile range
ITT	Intention-to-treat
Max	Maximum
Min	Minimum
n	Number of patients with an observation
SAP	Statistical Analysis Plan
SD	Standard Deviation
SF-36	Short form health survey – 36 questions
TGN	Thioguanine
TOPPIC	Trial acronym for 'Randomised Controlled Trial of 6-Mercaptopurine Versus Placebo to Prevent Recurrence of Crohn's Disease Following Surgical Resection'
TPMT	Thiopurine s-methyltransferase

3 Introduction

This document details the criteria to be used for the definition of the analysis populations and the statistical methodology for analysis for the TOPPIC trial, a randomised trial of 6-mercaptopurine versus placebo to prevent recurrence of Crohn's disease following surgical resection. This document has been compiled according to the Edinburgh Clinical Trials Unit (ECTU) standard operating procedure (SOP) "Statistical Analysis Plans" and has been written based on information contained in the study protocol version 13, dated 29 April 2015.

TOPPIC is a multicentre, randomised, parallel-group, placebo-controlled trial. Treatment allocation is a 1:1 ratio. Patients are randomised to either 6-mercaptopurine or matched placebo control. The aim was to recruit 234 patients.

4 Statistical Methods Section from the Protocol

The primary outcome variable is postoperative recurrence of Crohn's Disease and it's timing if it recurs. Analysis will be by intention-to-treat and will be based on the application of Cox proportional hazards model. The primary analysis will include terms for the treatment, the variables on which the randomisation was stratified and adjusted for baseline values of previous treatment with 6MP and previous treatment with Azathioprine.

The quality of life variables will be analysed using a repeated measures analysis of covariance to evaluate treatment and treatment by time interactions.

The use of faecal calprotectin as a non-invasive marker of disease recurrence will be examined in two ways. It will be firstly considered as a time dependent covariate in the Cox proportional hazards model described above. Secondly, levels will be compared descriptively between those with negative or positive colonoscopies at 12 and 36 months. Similarly, 6MP drug metabolite levels will be also considered as a time dependant covariate in the Cox proportional hazards model.

Exploratory analyses will investigate the inclusion of the clinical, genetic and serological markers in the model. The models will be used to develop a simple scoring system to predict the risk of recurrence. The sample size will be too small to use a split sample approach to validate the prediction, but the use of a simple scoring system will mitigate to some degree the more extreme effects of using observed regression coefficients to define the risk. During the period after trial recruitment, data from new patients will provide limited short-term validation of the predictions.

5 Overall statistical principles

Health economic analyses will not form part of the statistical report.

All analyses will be intention to treat (ITT), except analyses of safety reporting, where patients will be analysed according to the treatment they received. The ITT population will include all patients who have been randomised into the TOPPIC study. Patients will be analysed in the group to which they were randomised, regardless of treatment received. For analysis according to treatment received, patients will be in the 6-MP group if they receive any 6-MP tablets, and the placebo group if they did not receive any 6-MP tablets.

In general terms, categorical data will be presented using counts and percentages, whilst continuous variables will be presented using the mean, median, standard deviation (SD), minimum, maximum, inter quartile range (IQR) and number of patients with an observation (n), using the format presented in the examples 1 and 2 below. Data will be split by timepoint where applicable.

Example 1. Data presentation for categorical data

Parameter	Timepoint / visit	Statistic/ category	**6-MP** N=xx	**Placebo** N=xx	**Overall** N=xx
Parameter A	Timepoint x	Category 1	xx (%)	xx (%)	xx (%)
		...	...	...	...
		Category n	xx (%)	xx (%)	xx (%)

Example 2.Data presentation for continuous data

Parameter	Timepoint / visit	Statistic/ category	**6-MP** N=xx	**Placebo** N=xx	**Overall** N=xx
Parameter	Timepoint x	Mean	xx	xx	xx
		Median	xx	xx	xx
		SD	xx	xx	xx
		Q1, Q3	xx, xx	xx, xx	xx, xx
		Min, Max	xx, xx	xx, xx	xx, xx
		n	xx	xx	xx

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. 95% (2-sided) confidence intervals (CIs) will be presented. All analyses are seeking to show a difference, rather than equivalence or non-inferiority.

Where there is missing data for an outcome variable, in the first instance, those records will be removed from any formal statistical analysis relating to that outcome variable, unless otherwise specified. In tabulations, numbers of missing observations will be provided, but percentages will not include them.

The main statistical analyses for primary and secondary outcomes will be adjusted for centre and smoking status (from randomisation stratification) and baseline values of 'Previous treatment with 6MP' and 'Previous treatment with Azathioprine' following note from DMC in January 2013. Results of unadjusted statistical analyses will also be presented.

Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of residual plots. Normality will be examined by normal probability plots. If the distributional assumptions for the parametric approach are not satisfied, further data transformation (to alleviate substantial skewness (i.e. normalizing) or to stabilise the variance), or other suitable methods will be considered. This will be documented in the statistical results report together with the reasoning supporting the action taken, if applicable.

All analyses and data manipulations will be carried out using SAS [1].

6 List of analyses

6.1 Recruitment and retention

The date of first and last patient randomised, the number of patients randomised, and the number of centres that recruited patients, will be reported.

A CONSORT flow chart will be provided by the TOPPIC Trial Manager. This will show the number and percentage of patients considered for randomisation, randomised, dosed, completed and discontinued by treatment and overall. Reasons for non-inclusion in the study (prior to randomisation) will be categorised, as in the data monitoring committee (DMC) reports. The number of patients discontinued early from the study will be summarised by reason for withdrawal and treatment.

6.2 Baseline Balance

No formal statistical testing will be performed. The following will be presented and summarised by allocated treatment:

- Consent obtained
- Variables from randomisation stratification: Centre, Smoking status. Smokers will be defined as those smoking >1 cigarette/day
- Other data recorded at pre-assessment and randomisation visits:

Pre-assessment

Gender
Previous treatment with:
6MP/Azathioprine/Other corticosteroids
Infliximab or Methotrexate
No immunosuppressants
Crohn's disease location & type
Previous surgery
TPMT
Age at randomisation
Age at diagnosis
Duration of Crohn's disease

Randomisation/Visit 2

Weight, Height
Faecal calprotectin
Neutrophil count
CDAI score
IBDQ/EQ-5D/SF-36

6.3 Adherence with trial protocol and allocated treatment

No formal statistical testing will be performed. The following will be presented:

- Number and percentage of patients who were ineligible for inclusion in trial (if any – it should not have been possible to randomise such patients).

- Numbers of patients who were randomised but never treated.

- Numbers of patients who took trial medication for the full 3 years, and those who stopped early.

  For those who stopped early, they will be categorised as:

  Primary outcome-related event[1]
  Other adverse event
  Blood test result
  Other reasons

  Time to stopping medication will be presented as a Kaplan-Meier plot, split by allocated treatment.

- Prescribed dose will be summarised. Dose at final visit compared with dose at first visit will be summarised as:

  Dose increased
  Dose stayed the same
  Dose decreased
  Trial medication stopped early

- A summary of patients attending and withdrawing at each visit will be presented, together with reasons for withdrawal.

- A listing of patients where the blind was broken early will be presented, including details of allocation, timing and reason for breaking blind, and outcome.

6.4 Concomitant Medication

Number and percentage of patients will be presented by medication, treatment and overall, in the categories used in the database. These will be split into prohibited medications, and non-prohibited medications.

For non-prohibited meds, these will be split into those used as rescue therapy, and those used for other reasons. For prohibited meds, the number of patients who take each medication type (5 ASAs, corticosteroids, anti–tumour necrosis factor, azathioprine, methotrexate, allopurinol, antibiotics for Crohn's disease, oral non-steroidal anti-inflammatory drugs) will be tabulated, split by whether these are rescue meds or not.

[1] Postoperative clinical recurrence of Crohn's disease (defined by a Crohn's Disease Activity Index value of greater than 150 together with a 100 point rise in the CDAI score from baseline), together with the need for anti-inflammatory rescue therapy or primary surgical intervention.

6.5 Primary Outcome

The primary outcome variable is the timing of postoperative clinical recurrence of Crohn's disease[2].

For the purposes of the statistical analysis, the date of recognition of clinical recurrence as assessed by the CDAI score will be considered the date of primary outcome provided that anti-inflammatory rescue therapy or primary surgical intervention is initiated as part of the same clinical episode. If there is any doubt that events form part of the same clinical episode, this will be assessed by the CI who will be blinded to the treatment allocation. It is recognised that, in some cases, rescue therapy may have been initiated before the CDAI score reaches the trigger criteria.

The primary outcome will be assessed as follows:

a. The times, split by treatment group, will be presented using a Kaplan-Meier plot. If distributional assumptions hold, this will be analysed using Cox proportional hazards regression. The effect of treatment allocation will be reported as an adjusted (as detailed in Section 5) hazard ratio with its corresponding 95% confidence interval. Unadjusted results will be presented as a secondary analysis.

b. Length of patient follow-up will be summarised, together with a summary of the number followed for 3 years or having a recurrence.

6.6 Secondary outcome

1. A secondary outcome variable of clinical recurrence of Crohn's disease (defined by a CDAI value of greater than 150 together with a 100 point rise in the CDAI score from baseline), OR the need for anti-inflammatory rescue therapy OR primary surgical intervention will be analysed in the same way as the primary outcome.

   The number of patients with each element of this combined endpoint will be summarised separately. For rescue therapy, the number of patients who take each medication type (5 ASAs, corticosteroids, anti–tumour necrosis factor, azathioprine, methotrexate, allopurinol, antibiotics for Crohn's disease, oral non-steroidal anti-inflammatory drugs) will be tabulated in the Concomitant Medication section. For surgical intervention, the number of surgical interventions per patient will be summarised. These will be classified as primary or secondary. The time to surgery will also be summarised.

   a. An informal sensitivity analysis comparing patients who are 'on-drug' versus those who are 'off-drug' at the end of their time in the trial with respect to the secondary endpoint will be presented by means of a Kaplan-Meier plot. No formal statistical testing will be performed.

[2] Postoperative clinical recurrence of Crohn's disease (defined by a Crohn's Disease Activity Index value of greater than 150 together with a 100 point rise in the CDAI score from baseline), together with the need for anti-inflammatory rescue therapy or primary surgical intervention.

2. Endoscopic recurrence using both the Rutgeert's and CDEIS scoring system will be summarised by time and treatment group. Colonoscopy results[3] will also be summarised.

3. Colonoscopy results at Visit 12 will be compared between the treatment groups using a chi-squared test. CDEIS scores at Visit 12 will be compared between the treatment groups using a t-test.

4. Faecal calprotectin results will be summarised by time and treatment group, both as a continuous measure and categorically. The continuous change from baseline to each time point will also be summarised.

   The use of faecal calprotectin as a non-invasive marker of clinical recurrence of Crohn's disease[4] will be examined in two ways:

   i. Levels will be compared descriptively between those with negative (≤ i1) or positive (≥ i2) colonoscopies at 12 and 36 months.

   ii. Time to disease recurrence will be analysed using a Cox proportional hazards model (as detailed in Section 6.5), incorporating calprotectin results as a time dependent covariate, provided there are sufficient outcome events.

   Hazard ratios with corresponding 95% confidence interval will be estimated, indicating whether calprotectin levels are surrogate markers of disease recurrence.

5. Thioguanine (TGN) levels of the 6MP drug metabolite samples will be summarised by time and treatment group, both as a continuous measure and categorically following independent blind review. The continuous change from baseline to each time point will also be summarised.

   TGN levels of the 6MP drug metabolite samples will also be considered as a time dependant covariate in the Cox proportional hazards analysis of time to disease recurrence (as detailed in 3.ii above). Patients receiving placebo will not be included in this analysis.

6. Quality of life will be assessed by the validated indices IBDQ, EQ-5D and SF-36, as follows:

   a. The values at baseline and each time point, in each of the various subscales of these measures will be summarised (without statistical testing). The change from baseline to each time point will be summarised for IBDQ (average and total) and SF-36 only (physical component score and mental component score).

   b. Change from baseline in overall IBDQ (average and total) and SF-36 (physical component score and mental component score) indices will be

[3] A negative colonoscopy is defined as a Rutgeert's score of ≤ i1, while a positive colonoscopy is defined as a Rutgeert's score of ≥ i2.

[4] The primary outcome i.e. a Crohn's Disease Activity Index value of greater than 150 together with a 100 point rise in the CDAI score from baseline, together with the need for anti-inflammatory rescue therapy or primary surgical intervention.

modelled using a repeated measures analysis of covariance, fitting terms for treatment, time (visit) and the interaction between treatment and time (visit). Patient and time will be included in the model as random effects.

The estimated treatment effect and 95% confidence intervals will be presented at each timepoint and overall.

c. IBDQ takes precedence over the other Quality of Life scales in terms of clinical importance. In order to take account of missing response data, a sensitivity analysis using multiple imputation techniques may be undertaken for IBDQ only. This will be analysed in the same way as the proposed repeated measures analysis (4b).

6.7 Safety analyses

1. Adverse Events (AEs) will be summarised by treatment and by severity, causality and seriousness. No formal statistical analysis will be performed. A listing will be produced detailing each event, and what happened to the patient subsequently.

2. Details will be provided of any patients who become pregnant or who have a partner who becomes pregnant during the study. A listing will be produced detailing each event, and what happened to the pregnancies subsequently.

3. Blood safety monitoring tests of interest will be summarised by treatment and visit. Abnormal laboratory results outside of the normal reference range will also be summarised. Results of particular interest are: ALT, Alk Phos, Albumin, CRP, WBC, haemoglobin, neutrophils and lymphocytes.

6.8 Subgroup analyses

The following subgroup analyses are planned:

i. Patients who are thiopurine naïve (have had previous treatment with either 6MP or Azathioprine)

ii. Patients who have had previous treatment with Infliximab or Methotrexate

iii. Patients who have had previous surgery

iv. Smoking status

v. Duration of disease (≤ 1 year, > 1 year)

vi. Age at diagnosis (≤40 years, > 40 years)

The primary and secondary outcomes defining clinical recurrence of Crohn's disease will be analysed for these subgroups. The interaction between subgroup and treatment will be included in the Cox regression model (as described in Section 6.5) to determine if the treatment effect differs by subgroup.

Endoscopic recurrence as defined by Rutgeert's score and the CDEIS score will be summarised and analysed separately for these subgroups in line with the methods described in Section 6.6. Similarly to the subgroup analyses of the primary and secondary outcomes, the interaction between subgroup and treatment will be assessed to determine if the treatment effect differs by subgroup.

6.9 Exploratory analyses

Exploratory analyses will be undertaken to investigate the inclusion of clinical, genetic and serological markers as a means of predicting the risk of disease recurrence.

A simple scoring system will be devised by implementing a predictive model which will utilise the markers available.

The number of terms to go into the model will be determined based upon the number of outcome events (i.e. disease recurrence) observed. It is recommended that approximately ten outcome events are observed per explanatory variable for it to merit inclusion in a predictive model.

Appropriate explanatory variables will be selected based on clinical relevance, precision of measurement, and amount of missing data. Correlation between possible explanatory variables will also be considered in order to avoid the same underlying effect being measured by two or more variables.

The model will be evaluated in terms of calibration (i.e. how closely predicted outcomes match actual outcomes) and discrimination (i.e. how well the model distinguishes between low and high risk patients).

This exploratory analysis will not form part of the primary statistical report, but will follow in a separate subsequent report.

7 Validation

The following will be done by a second statistician:

1. Separate programming and checking of primary outcome results and conclusions.

2. The statistical report will be read and sense-checked.

8 Data sharing

A file, or set of files, containing the final data will be prepared, along with a data dictionary. These will be made available to the Chief Investigator at the end of the analysis phase.

9 References

1. SAS® Institute Inc. SAS for Windows. SAS Institute Inc.: Cary, NC, U.S.A