STATISTICAL ANALYSIS PLAN

IST-3

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1. Introduction

Thrombolytic therapy with intravenous recombinant tissue plasminogen activator (rt-PA) is approved for use in Europe and other countries for the treatment of selected patients with acute ischaemic stroke. The EU approval specifies a number of selection criteria: the patient should be aged less than 80 years, meet a number of other criteria, be assessed and have treatment initiated within three hours (this 'time window' is expected to increase to 4.5 hours in the light of the ECASS-3 trial results[1]). However, a systematic review of trials of thrombolysis for patients with acute ischaemic stroke has suggested that thrombolysis is very promising for patients who can be treated up to 6 hours after stroke onset.[2] There is a lack of data and a lack of expert consensus regarding the effects of thrombolysis for acute ischaemic stroke among patients who do not exactly fulfil the criteria of the EU approval and so the balance of risk and benefit for many types of patient remains unclear.

There is clear evidence from a variety of sources on several aspects of thrombolytic therapy with rt-PA:

- the odds of a favourable outcome with treatment decline steeply with time, and so, the earlier treatment is given, the better [3]
- treatment is associated with a clinically and statistically highly significant excess of fatal intracranial haemorrhages[2]
- The presence of ischaemic change[4] on the pre-treatment scan is an adverse prognostic factor (for survival free of dependency), and patients with more extensive or more marked ischaemic changes may also be at higher risk of symptomatic intracranial haemorrhage[5]

Furthermore, blinded analyses (of both treatment groups combined) of the baseline characteristics of the patients recruited in IST-3 identified trends in key prognostic factors among patients recruited at different times after stroke onset that might complicate the assessment of the effects of treatment overall and in particular subgroups.[6] These trends indicated the need to revise the existing analytic strategy before the data were unblinded.

2. Study objectives

The Third International Stroke Trial (IST-3) is a large-scale pragmatic trial conducted in order to assess the balance of risk and benefit more precisely for thrombolytic therapy with rt-PA. The pragmatic design means that the trial has broad entry criteria to maximise the generalisability of its results.[7, 8] The trial seeks to:

- determine whether a wider variety of patients than previously thought might benefit from this treatment;
- assess which categories of patients are most likely to benefit (by investigating the interaction between treatment effect and: age; stroke severity; early brain imaging appearances; other clinical features);
- refine current estimates of the duration of the 'therapeutic time window';
- improve the external validity and precision of the existing estimates of the overall treatment effects (efficacy and safety).

The primary trial hypothesis is that intravenous recombinant tissue plasminogen activator (rt-PA) in a dose of 0.9mg/kg (maximum 90mg) administered to patients with acute ischaemic stroke, within six hours of symptom onset, increases the proportion of people alive and independent at six months.

3. Purpose of the Statistical Analysis Plan

Full details of the background, design and operation of IST-3 are documented in the trial protocol and the operations manual (for current versions, see www.ist-3.com). The main features of the trial are described briefly in Section 4. The history of the study, protocol amendments during course of the study and the baseline characteristics of the patients included in the study have been reported separately.[6] The purpose of this Statistical Analysis Plan is to provide a clear definition of the main analyses to be reported in the primary report of the trial results. The nature of further secondary analyses and content of subsequent publications cannot be specified in detail, but where appropriate, we set out the general analytical approach. The Statistical Analysis Plans for the IST-3 advanced stroke imaging study (cerebral perfusion and cerebral angiography) will be reported separately.

4. Study design

The study is an international multi-centre randomised trial. The initial phase of the trial had a double blind design, patients were to be allocated rt-PA (0.9mg/kg) or matching placebo, and up to 300 patients were to be included (276 patients were randomised in this phase). All subsequent patients were included in the open phase of the trial. In the open phase, the patients and treating clinicians were not blinded to treatment allocation, but outcome assessment was, as far as possible, blinded to treatment allocation. Both arms were managed in the same environment according to local acute stroke care protocols. Assessment of the primary outcome at six months was

by either (i) postal questionnaire completed by the patient or an appropriate proxy; or (ii) blinded telephone interview; or (iii) a clinic assessment by a clinician blinded to treatment allocation, who was not involved in the patient's acute treatment. The expert scan readers were blinded to all clinical details, treatment allocation and whether the scan was pre- or post-randomisation. A full list of the centres participating in the trial, analysis of the baseline characteristics of the included patients, the funding sources and sponsors and the most recent version of the protocol are available at: http://www.trialsjournal.com/content/12/1/252

4.1 Eligibility

Patients with mild, moderate or severe strokes were potentially eligible if the following criteria were met:

- Symptoms and signs of clinically definite acute stroke.
- Time of stroke onset is known and treatment could be started within six hours of this onset.
- CT or MRI brain scanning had excluded both intracranial haemorrhage and structural brain lesions that can mimic stroke (e.g. cerebral tumour).

A detailed list of exclusion criteria is contained in the protocol (www.ist-3.com). Briefly, the trial excluded patients who were dependent in activities of daily living[a]; had a recent history of stroke; had known coagulation or platelet defects; were hyper- or hypo-glycaemic; or hyper-/hypotensive.

The protocol emphasised that patients should not be randomised if there was a clear indication for, or a clear contraindication to, thrombolytic therapy. If the clinician felt, for that particular patient, treatment was **promising** but unproven, and if the patient or relevant proxy gave consent, the patient was eligible for inclusion.

4.2 Randomisation

Patients were entered into the trial by telephoning a fast, secure computerised central randomisation system or via a secure web interface. Allocation to rt-PA or control was decided by a minimisation algorithm. The study centres were stratified into eight world regions (North-west Europe, Scandinavia, Southern Europe, Eastern Europe, Australasia, Americas, Asia and Rest of world; in fact only the first five of these regions had significant recruitment). Within each region, the algorithm balanced the number of patients in each arm of the trial according to the following variables: age (>= or < 70); sex; NIH stroke score (0-5, 6-10, 11-15, 16-20 or >20); time from onset to randomisation (<= or > 3 hours); use of antiplatelet agents within 48 hours pre-randomisation (Yes, No or Unknown); and stroke subtype (LACI or other). The system used a minimisation program to achieve optimum balance within centres for these key clinical prognostic factors, and from January 2006, the minimisation algorithm was additionally stratified by world region and minimised on everything else within world region. Because simple minimisation within centres can lead to alternation of treatment allocation and thus potential loss of allocation concealment, the system also incorporated a degree of random allocation - i.e., the minimisation algorithm was used to allocate patients with a probability of 0.80 to treatment or control group.

4.3 Brain Imaging

All patients were to have a brain scan before randomisation (to exclude intracranial haemorrhage and non-stroke lesions as the cause of the symptoms) and a follow-up scan at 24-48 hours. In addition a repeat scan was required if the patient deteriorated neurologically or intracranial haemorrhage was suspected for any reason. Although CT scanning was preferred, MR brain imaging was allowed. All scans were sent to the trial centre in Edinburgh where they were digitised if necessary and coded. Images were assessed with all original identifiers stripped from the record, and then viewed via a secure web-based image viewing system by an international panel of expert radiologists. All assessments were made blind to all patient details and treatment allocation.

4.4 Infusions

Patients allocated to rt-PA were given a total dose of 0.9mg per kg of body weight up to a maximum of 90mg. Ten per cent of the dose was given as an intravenous bolus delivered over a few minutes followed by the rest of the infusion over the next 60 minutes. Patients allocated to control received stroke care in the same clinical environment as those allocated to rt-PA, but had to avoid treatment with rt-PA.

4.5 Blood pressure monitoring

[a]In the initial double-blind phase of the study, the protocol permitted patients who needed minimal help in activities of daily living (and therefore not fully independent) to be included in the study. However, after 17 such patients had been included, the protocol was modified and no further such patients were included in the study.

The IST-3 protocol did not specify an algorithm for blood pressure management. The protocol required that blood pressure be managed according to local guidelines. Blood pressure was recorded for trial purposes at randomisation, start of infusion, 30 minutes into the infusion, at the end of the infusion (at 1 hour), and 24 hours after the infusion and was recorded on the treatment and monitoring form. In the open phase of the trial, patients allocated control had blood pressures recorded immediately after randomisation, at 30 & 60 minutes and 24 hours after randomisation. Data on the use of blood pressure lowering drugs between randomisation and day 7 were collected on the seven day form (see section 4.6).

4.6 Follow up in first seven days

The first follow-up was due at seven days, hospital discharge, transfer to another hospital or death, whichever occurred first. The Hospital Co-ordinator at each collaborating centre completed the hospital follow-up form for each patient. Information was collected on pre-and post-admission treatment, the clinician's final diagnosis of the initial event leading to randomisation, details of all post-admission cerebral and other events (including adverse reactions). For patients who died before day 7, the date and likely cause of death were noted. For patients who were alive at day 7, their functional status was recorded: Glasgow Coma Scale, ability to walk without assistance, ability to lift both arms off the bed and whether or not the patient was judged to be independent in activities of daily living. All patients were followed up, whether or not they complied with their treatment. In some centres, where advanced imaging techniques were performed as part of routine clinical care, the cerebral perfusion and angiography images were also sent to the trial coordinating centre. The analysis of the data from these advanced imaging techniques will be reported separately. In Sweden and Norway, a separate sub-study collected health economic data; this will also be described separately.

4.7 Follow up at six months

Six months after randomisation, General Practitioners or Hospital Co-ordinators were contacted to check whether the patient was alive and could be approached for follow-up. If appropriate, a self-completion questionnaire was mailed to the patient, to record dependency and health related quality of life. In Italy, patients were followed by structured telephone interview conducted by a highly experienced interviewer, blinded to treatment allocation. In Portugal, six month follow-up was conducted in person by a clinician who was not involved in the patient's treatment, blinded to their treatment allocation. If a patient was still in hospital at six months, a similar questionnaire was completed by hospital staff. In Sweden, all patients were followed up by postal questionnaire mailed from the National Co-ordinating Centre, and for those that did not respond, by structured telephone interview conducted by the trial monitor. If a patient died after a seven day follow-up form had been completed, and within 6 months of randomisation, the clinician could complete and return a simple form to the IST-3 Trial Office so as to reduce the risk of the co-ordinating centre mailing a questionnaire to a patient who had died. Every effort was made to determine the precise date of death for survival analyses. If a patient withdrew consent for follow-up or was not traceable, the patient's survival time was censored at the last known contact date. Every effort was made to determine the reason for loss to follow-up, and in fact such losses were minimal.

4.8 Follow up at eighteen months

In a subset of countries, including the UK and Australia, patients were followed up again at eighteen months. This follow-up was by postal questionnaire similar to the six month follow-up, and by telephone follow-up for non-responders. These data were collected to permit more detailed health economic modelling and to test the hypothesis that the level of disability at six months predicted longer-term survival.

4.9 Definition of outcomes

4.9.1 Primary outcome

The primary measure of outcome is the proportion of patients alive and independent six months after randomisation. The protocol states that the modified Rankin Scale (mRS)[9] will be used for the assessment of functional outcome at six months. There are several formats and derivatives of the Rankin Scale and of the mRS, each with slightly different wording and implementation.[10] The questionnaires that were used in IST3 employed the wording of the 1990 version of the mRS,[11] which was slightly modified for use as a postal questionnaire (this version of the mRS has also been referred to as the Oxford Handicap Scale). The OHS version of mRS has been widely used in stroke research studies and correlates well with other measures of functional capacity in stroke patients[12, 13] The exact wording used in the trial questionnaires is shown in Appendix 4. Patients responding 'no symptoms', 'few symptoms', 'some changes… but still able to look after myself' were defined as independent. The first published version of the mRS (van Swieten 1988[9]) differentiates grade 3 from grade 4 by the ability to walk (grade 3 = able to walk without assistance, grade 4 = needs assistance to walk).To allow a degree comparability with studies that have used the van Swieten version of the mRS, the outcome assessment included the question:

'Do you need help from anybody to walk? The six month form included several other questions related to activities of daily living (see Appendix 4).

4.9.2 Secondary outcomes
For each patient who was reported to have deteriorated clinically or to have developed symptoms suggesting that a new cerebral event had occurred within seven days of randomisation, an adjudication committee reviewed selected data from the seven day form and the expert panel's blinded reading of any brain images taken between randomisation and day seven. The adjudication committee were provided data on the date and time of randomisation, the patients OCSP stroke syndrome (TACI, LACI, PACI, POCI) at entry, the date and time of any event and the suspected type of event (see table 2 for the list of events), and a detailed report of the expert's opinion of any post-randomisation images. These data were reviewed blinded to the treatment allocation, and other patient baseline clinical data. If the patient was dead by day seven, one of the following death categories was assigned:

1. Death from initial stroke attributed to infarct swelling.
2. Death from initial stroke attributed to intracranial haemorrhage.
3. Death from initial stroke not attributable to infarct swelling or symptomatic intracranial haemorrhage.
4. Death due to recurrent ischaemic stroke.
5. Death due to recurrent stroke of unknown type.
6. Death due to non-cerebral causes.

If the patient was alive on day seven but had had a non-fatal cerebral event between randomisation and seven days while in hospital, one of the following categories was assigned:

7. Neurological deterioration, attributed to swelling of initial ischaemic stroke.
8. Symptomatic intracranial haemorrhage.
9. Neurological deterioration not attributable to brain swelling or symptomatic intracranial haemorrhage.
10. Recurrent ischaemic stroke.
11. Recurrent stroke of unknown type.

Detailed definition of each of the above categories is contained in the SOP for the event adjudication committee (see Appendix 2). Other non-fatal, non-cerebral events in hospital in the first seven days were also recorded, and were subject to coding and consistency checks by the trial data management team.

5. Statistical Analysis
All analyses will be by intention to treat. By this, we mean that patients will be analysed in the group they were randomised to, no matter what treatment they received, and regardless of whether they deviated from the protocol in any way. If it happens that a patient was mistakenly randomised more than once, only the first randomisation will be used. An exception was to be made for analyses of suspected unexpected serious adverse reactions (SUSARs), where patients were to be analysed according to the treatment they received, as this analysis is the most conservative.[b]

5.1 Basic characteristics
In order to assess balance, the rt-PA and control groups will be tabulated with respect to the following variables (Table 1): age; sex; NIH stroke score; time from onset of stroke symptoms to randomisation; stroke subtype; blood pressure and blood glucose at randomisation; randomising doctor's opinion of brain scan at randomisation; key variables from the expert panel interpretation of the brain scan; history of previous stroke; pre-admission treatment for hypertension and diabetes; pre-admission use of anti-platelet agents, heparin and warfarin, whether the centre had previous experience of thrombolysis for acute stroke, world region, and estimated probability of being alive and independent at six months as derived from the model of Konig based on age and NIH stroke score.[14] Some of these variables were used in the minimisation algorithm determining randomisation allocation and good balance would thus demonstrate successful operation of the algorithm.

5.2 Primary outcome: functional status at 6 months

5.2.1 Missing values in assessment of function at 6 months
If functional status at 6 months is unknown for any patient, we will apply the following algorithm. If the patient was alive at 6 months and measurements are available after baseline, we will use the level of function recorded on the

[b] As only one SUSAR occurred in the course of the trial, this strategy will not be applied.

7 day form (i.e. measured at 7 days or at prior discharge from hospital) to impute 6 month functional status. The early outcome form records whether the patient is independent in activities of daily living at 7 days, whether they can walk without assistance and lift both arms. Hence, 6 month OHS will be imputed for patients with status at 7 days (see table below). We have chosen this simple form of single imputation, as it classifies well patients for whom both 7 day and 6 month data are known, and as any additional gain from more complex multiple imputation methods is likely to be small[15]

Status at 7 days			Imputed 6 month OHS
Independent Y/N	Able to walk Y/N	Able to lift arms Y/N	
Y			2
N			5
Missing	Y	Y	2
Missing	N	N	5
Missing	Missing	Missing	5

5.2.2 Main analysis of primary outcome

Table 2 shows the planned presentation of the primary outcome. The numbers in each treatment group with known vital and known or imputed disability status at six months will form the denominators of the primary outcome percentages; it is expected that these denominators will be very close to the numbers randomised. We will also perform a sensitivity analysis restricted to patients with known disability and vital status (webtable 2). The analysis of the baseline characteristics of the patients in the trial showed clear trends in key prognostic factors (age, stroke severity, degree of ischaemic change on baseline CT/MR) among patients randomised at different times after stroke onset that might complicate the estimation of the effect of treatment overall and in subgroups.[6] The primary analysis of the effect of treatment on the primary outcome will therefore be adjusted for the following covariates: age; NIH stroke score; time from onset of stroke symptoms to randomisation; presence (vs. absence of) of ischaemic change on the pre-randomisation brain scan according to the expert read;[c] an unadjusted analysis will also be presented. A secondary analysis will be undertaken using ordinal logistic regression, with the OHS as dependent variable. We will reduce the OHS from 7 to 5 levels, analysing levels 0,1,2,3 separately and combining levels 4, 5 & 6 into a single level. If such analysis suggests notably different conclusions from the analysis based on dichotomous outcome, reasons for the differences will be explored in secondary analyses to be published subsequent to the primary paper. The effect of treatment allocation on survival will be described by presenting a Kaplan-Meier survival plot (Figure 5).

5.2.3 Key subgroup analyses of the effect of treatment on primary outcome

In view of the history of the trial and the state of knowledge before the trial results are analysed, the primary pre-specified subgroup analyses are particularly important. When the trial began recruitment, rt-PA was not approved for use in Europe for the treatment of stroke. However, during the course of the trial, EMEA approved rt-PA for use in certain patients with acute ischaemic stroke. Investigators did not include patients in the trial who were suitable for treatment with rt-PA and hence the majority of trial patients included in the trial do not meet the criteria set out in the EMEA approval.[6]

We have adopted the broad approach recommended by Kent, to specify a small number of primary subgroups and to include analyses stratified by baseline risk (of a poor outcome); these are listed below.[16] We will also report a subgroup analysis of the effect of treatment on the primary outcome, subdivided by the predicted risk of a poor outcome derived with the model of Konig[14] from the patient's age and their baseline NIHSS.

The primary pre-specified subgroups are defined by:

- age
- time from stroke onset to randomisation
- initial stroke severity as measured by NIH stroke score,
- appearance of the baseline brain scan (whether ischaemic change is visible or not) on expert read

[c] If the baseline scan was not available for blinded central assessment by the expert panel, the analysis will use the randomising clinician's pre-randomisation assessment of the scan (which had to be entered in the randomisation system before the patient's treatment allocation was revealed).

These were selected after review of factors that are predictors of prognosis, and for which there is prior evidence that they are potentially important effect modifiers. Over half of IST-3 patients were aged over 80 (the upper age limit for licensed use of rt-PA) and nearly three-quarters were randomised more than three hours after stroke onset (the upper time limit for licensed use).[6] It is anticipated that any relative benefit in the primary outcome will be smaller for subgroups which meet any of the following criteria: older age, longer delay times between stroke onset and randomisation, greater initial stroke severity, or had ischaemic change visible on the pre-randomisation scan. Therefore the evidence for variation in rt-PA treatment effect across subgroups defined by these variables (including predicted risk of dependence, which is based on age and NIH stroke score) will be interpreted without any consideration of multiple testing. The interpretation will depend on the p-value for interaction, and the size and confidence limits for the effects in the subgroups being compared. However it is not anticipated that IST-3 will have sufficient power to detect small differences in rt-PA treatment effects between subgroups. A planned individual patient meta-analysis (see section 5.7) including data from IST-3 and other relevant trials will have greater power in this regard.

The 2-way interactions of rt-PA treatment effect with age, NIH stroke score, time to randomisation, presence /absence of visible ischaemic change on the pre-randomisation scan (as determined by the expert panel) on the primary outcome will be explored through multivariate logistic regression. For each treatment by subgroup interaction, the change in log likelihood when the interaction term is added to a logistic regression model containing the treatment and subgroup main effects will be calculated. The significance of the interaction will be assessed by comparing the change in log likelihood with percentage points of a chi-squared distribution with the appropriate degrees of freedom (a likelihood ratio test). Where a factor has more than two levels the test is for the null hypothesis that all levels have the same underlying odds ratio versus the alternative that the odds ratios have a linear trend (if the levels are ordered), or simply that the odds ratios are not all equal (if the levels are not ordered). The cutpoints for continuous variables have been chosen by reference to an analysis of baseline characteristics (both treatment groups combined) so as to maximise power. Forest plots will be constructed to illustrate subgroup analyses.

5.3 Secondary outcomes

5.3.1 Secondary outcomes at seven days

Table 3 shows the planned presentation of secondary outcomes occurring within seven days. These will be adjusted in the same fashion as the primary outcome. Unadjusted analyses will be presented.

- all deaths due to cerebral events
- all deaths due to specified non-cerebral events (myocardial infarct, extracranial bleed, allergic reaction)
- all deaths
- each type of fatal cerebral event
- each type of non-fatal cerebral event
- each type of non-fatal non-cerebral event (myocardial infarct, extracranial bleed, allergic reaction)
- total fatal and non-fatal cerebral events
- total fatal and non-fatal cerebral events of each type
- total fatal and non-fatal non-cerebral events of each type

5.3.2 Secondary outcomes at six months

Secondary outcomes at six months are listed in appendix 3. Treatment effect on deaths from all causes and deaths from vascular causes will be assessed by Cox proportional hazards models. Treatment effect on health-related quality of life (EuroQol) will be assessed using a proportional odds model, supplemented with a Mann-Whitney test for surviving patients. Treatment effect on response to the simple recovery and dependency questions will be assessed using an ordered categorical model (with linear scores 0, 1 and 2 for 'Dead', 'No' and 'Yes'). Effects on residence will be assessed by a chi-squared test.

5.3.3 Secondary outcomes at eighteen months

Secondary outcomes at eighteen months are defined exactly as at six months, and will be analysed in the same way. Additionally change in health–related quality of life (EuroQol), and transitions between health status levels as defined by the OHS, and by the simple recovery and dependency questions will be analysed. We will impute a utility of 0 for those with missing values. These transitions will be treated as ordered categorical variables.

5.4 Additional subgroup analyses

The nature and extent of the exploratory analyses described below will be determined by the size and direction of the effects observed in the analysis of the primary outcome and main secondary outcomes, and cannot be specified in detail at this stage.

5.4.1 Subgroup analyses of the effect of treatment on the primary outcome
Each of the subgroups identified in Appendix 3 (tables 2 & 3), will be studied for interaction with the effect of rt-PA on the primary outcome, and, if appropriate additional analyses of subgroups defined by the criteria set out in Table 1. This will generate a large number of analyses, which can only be considered exploratory.

5.4.2. Subgroup analyses of the effect of treatment on the secondary outcomes
Each of the subgroups identified in Appendix 3 (tables 2 & 3), will be studied for interaction with the effect of rt-PA on each of the secondary outcomes identified in Section 5.3. This will generate a large number of analyses, which can only be considered exploratory. Particular interest will focus on the effect of rt-PA on the risks of symptomatic and fatal intracranial haemorrhage in the first seven days (as it is the single most important adverse effect of treatment with rtPA), to determine if there are important variations in the effect across the population of ischaemic stroke patients. It is anticipated that the risk of rt-PA in relation to intracranial haemorrhage will be larger for subgroups which are older, had longer delay times between stroke onset and randomisation, had greater initial stroke severity, and had ischaemic change on the pre-randomisation scan on expert read. Therefore the question whether age, delay time, NIHSS, or certain features of the baseline scan modify the treatment effect on the incidence of haemorrhage will be interpreted in terms of the significance (p values) of interactions without any adjustment for multiple testing. Other factors will also be explored, and the effect of treatment on the frequency of symptomatic haemorrhage according to definitions used in earlier trials (e.g. NINDS, and ECASSII), will be reported.in subsequent publications.

5.4.3 Additional analyses: imputing an 'onset to treatment time' for the open control group.
In the IST-3 trial, for patients recruited in the open phase of the study who were allocated control, it is not possible to specify a time interval from onset to 'treatment' that is comparable to the time from onset to delivery of the rt-PA bolus dose. However, to enable comparison with earlier placebo-controlled trials, we will perform a secondary analysis of the interaction between the 'imputed time from onset to treatment' and treatment effect. We undertook preliminary analyses to determine a simple and transparent approach to imputing this value for the control patients. For patients included in the open phase of the study, we analysed the determinants of the overall time to treatment in those allocated to rt-PA, and the contribution made by variation in the time from randomisation to the delivery of the bolus. As might be expected, variation in the delay from randomisation to delivery of the bolus (RTDB) was a small proportion of the overall delay from stroke onset to treatment. A multivariate model to predict RTDB accounted for only 10% of the variance in RTDB. We decided that, although imputing an RTDB for each individual patient derived from this model was possible, the marginal gain in accuracy was outweighed by the complexity and a certain lack of transparency. We therefore decided to impute RTDB delay by applying the mean delay of 18 minutes in all cases allocated control, so enabling a time from onset to treatment to be calculated for both treatment groups. We have chosen this simple form of single imputation, as the gain from more complex multiple imputation methods is likely to be small.[15]

5.4.4 Additional analyses: blood pressure post randomisation
We will plot systolic and diastolic blood pressure measured at 0, 30, 60 minutes and 24 hours after randomisation in each treatment group, and test for differences between blood pressures at each time point and overall between treatment and control.

5.4.5 Additional analyses: Detailed analysis of the expert readings of the brain images
Detailed analysis of the expert readings of the brain images are also to be undertaken, and likewise cannot be specified in detail at this stage, but may include analyses restricted to MCA territory infarcts so as to assess the role of the ASPECTS and 'one third MCA rule' appearance in modifying treatment effects. Influence of the appearance of the acute lesion (depth of ischaemia, lesion swelling, lesion extent, hyperdense artery) as well as of background brain features (atrophy, leukoaraiosis, prior vascular lesions) on risks and benefits with rt-PA, in the context of multivariable prediction models incorporating key clinical variables, will be evaluated.

5.5 Compliance and data quality analyses

5.5.1 Protocol deviations in consent procedure
These will be tabulated and accompanied by a brief textual description.

5.5.2 Protocol deviations in eligibility

If it emerges that any trial patients have been mistakenly included despite failing eligibility criteria (e.g. blood pressure, pre-randomisation anticoagulant treatment, features of pre-randomisation scan, or missing pre-randomisation scan), the numbers of randomised patients in each group failing each exclusion criterion will be tabulated (see webtable 1) but still included in the analysis. In the small number of cases where the original scan is unobtainable, and hence no expert opinion is available, the opinion of the local radiologist recorded in the medical records or the randomising doctor's opinion of the scan will be used, if appropriate.

5.5.3 Protocol deviations at infusion

The numbers of patients in each group receiving incorrect dose, incomplete infusion or no infusion will be presented (webtable 1). In the open phase it will be known whether or not patients received their allocated treatment, and if allocated thrombolysis, what dose was administered. It is possible that some patients allocated to thrombolysis will not receive their allocated treatment, and some of those allocated control will receive thrombolysis. These deviations will be tabulated. Patients will remain in their allocated treatment group for analysis, irrespective of treatment received.

5.5.4 Anti-thrombotic treatments in first 24 hours

The protocol precludes antithrombotic treatment in the first 24 hours after starting rt-PA treatment. This group should only receive such treatment after a second scan at 24-48 hours post- randomisation has excluded intracranial haemorrhage. However a small number of protocol deviations may occur, and the numbers in each group receiving aspirin, heparin or other antiplatelet agents in the first 24 hours will therefore be compared, with results presented separately for the blind and open trial phases (webtable 1).

5.5.5 Drug and other treatments in first seven days

The numbers and percentages of patients in each group receiving anti-thrombotic and other treatments such as antibiotics within the first seven days will be tabulated. The duration of stay in units with different levels of care (ICU, high dependency, acute stroke unit, stroke rehabilitation unit, general ward) will be tabulated (webtable 1) to ensure background treatment is comparable in the two treatment groups (this has been monitored by the Data Monitoring Committee during the course of the trial). This will permit a check on other factors that might affect the perceived balance of anti-thrombotic intensity between the two groups. Group differences on these factors will not be subjected to statistical tests, but in the event of differences for a given factor exceeding ten percentage points the factor will be included in a sensitivity analysis (Section 5.6).

5.5.6 Assessment of between-centre variations in data quality

Variation in completeness of data and median response time for submitting seven day follow-up forms will be assessed. Data from centres where the median exceeds the overall median by more than twice the semi-interquartile range of completeness or response times will be excluded in a sensitivity analysis.

5.6 Sensitivity analyses and multivariate modelling

5.6.1 Sensitivity analyses

In order to support conclusions, sensitivity analyses will be conducted by calculating p-values and confidence intervals for the treatment effect on the primary outcome after adjustment for (i) centre (taken as a random effect); (ii) country; (iii) region; (iv) region and all variables used in the minimisation algorithm described in section 4.2. These analyses will be performed using logistic regression and so treatment effects will be expressed as adjusted odds ratios. Sensitivity analyses to explore the effects of missing data for the primary outcome will also be undertaken. In the case that differences in background drug treatment (other aspects of background medical care) in the first seven days, or centres with significant data quality issues are identified (section 5.5.5 and 5.5.6) further sensitivity analyses will be run to assess whether adjustment for these factors affects the primary outcome. Similar analyses will be performed for the secondary outcomes described in section 5.3.

5.6.2 Modelling of effects on secondary outcomes

This work will be treated as exploratory. Modelling of the secondary outcomes will be deferred to later papers after analysis of the results for the primary outcomes as described above. Models will also be sought for intracranial haemorrhage within the first seven days and for all deaths before six months. Within constraints of time, modelling of all other secondary outcomes will be explored.

5.7 Systematic reviews and meta-analyses

The findings regarding the effects of treatment on the components of the primary outcome (death, survival with dependency, survival free of dependency), and the key secondary outcome, (symptomatic intracerebral haemorrhage) will be presented in the context of an update of the Cochrane systematic review[2] to give an overall meta-analytic assessment of the most important effects of thrombolysis with rt-PA. An individual patient data meta-analysis of all trials of rt-PA vs Control is also planned, to which IST-3 will contribute data.

6.0 Proposed format of data tables in main results publication

Table 1 Baseline Characteristics

	Rt-PA		Control	
	No.	**(%)**	**No.**	**(%)**
Baseline variables collected before treatment allocation[1]				
Region				
NW Europe (UK, Austria, Belgium, Switzerland)				
Scandinavia (Norway, Sweden)				
Australasia				
Southern Europe (Italy, Portugal)				
Eastern Europe (Poland)				
Americas (Canada, Mexico)				
Age				
18-50				
51-60				
61-70				
71-80				
81-90				
over 90				
Sex				
Male				
Female				
NIH Stroke Score				
0 to 5				
6 to 10				
11 to 15				
16 to 20				
21 to 35				
Time to randomisation				
0 to $\leq$ 3 hours				
> 3 to $\leq$ 4.5 hours				
$\geq$ 4.5 to 6 hours				
Cardiac rhythm				
Atrial fibrillation				
Sinus rhythm				
Systolic BP at randomisation (mm Hg)				
<=144				
145 – 164				
> =165				
Diastolic BP at randomisation (mm Hg)				
<=74				
75 - 89				
>= 90				
Blood glucose at randomisation (mmol/L)[2]				
<= 5				
6-7				
>= 8				
Treatment with antiplatelet drugs in previous 48 hours				
Yes				
No/unknown				

Table 1 (continued) baseline characteristics

Baseline variables collected before treatment allocation[1]	rt-PA No.	(%)	Control No.	(%)
Clinician's assessment of pre-randomisation scan				
No evidence recent ischaemic change				
Possible evidence of recent ischaemic change				
Definite evidence of recent ischaemic change				
Predicted probability of poor outcome at 6 months[3]				
< 0.4				
0.4 – 0.5				
0.5 – 0.75				
> 0.75				
Stroke clinical syndrome[4]				
TACI				
PACI				
LACI				
POCI				
Expert panel's blinded assessment of pre-randomisation scan[5]				
No evidence recent ischaemic change				
Definite evidence of recent ischaemic change				
Size of tissue lesion				
None				
Small				
Medium				
Large				
Very large				
Depth of tissue damage				
None				
Mild				
Severe				
Degree of swelling				
None				
Sulcal				
Ventricular				
Hyperdense Artery				
None				
Anterior				
Posterior				
Atrophy				
Yes				
No				
Periventricular lucencies				
Yes				
No				
Old vascular lesion				
Yes				
No				
Non stroke lesion				
Yes				
No				
Territory				
MCA or ACA or Borderzone				
Lacunar				
Posterior				
Indeterminate[6]				

Table 1 (continued) baseline characteristics

	rt-PA		Control	
Baseline features collected on 7 day form	**No.**	**(%)**	**No.**	**(%)**
Pre-trial history of stroke				
Yes				
No				
Pre-trial treatment with aspirin				
Yes				
No				
Pre-trial treatment with clopidogrel				
Yes				
No				
Pre-trial treatment with dipyridamole				
Yes				
No				
Pre-trial treatment with anticoagulants				
Warfarin or other oral anticoagulant				
Heparin[7] (low dose)				
Heparin[7] (full dose)				
None of the above				
Pre-trial treatment for hypertension				
Yes				
No				
Pre-trial treatment for diabetes				
Yes				
No				
Phase of trial in which patient recruited				
Double-blind				
Open				
Patients recruited in centre with pre-trial experience of thrombolysis for acute stroke[8]				
Yes				
No				

NIH = National Institutes of Health, TACI= Total Anterior Circulation Infarct, PACI = Partial Anterior Circulation Infarct, LACI = Lacunar Infarct, POCI = Posterior Circulation Infarct, MCA = middle Cerebral Artery, ACA = Anterior Cerebral Artery

1. These variables were collected via the web-based or telephone randomisation system and had to be entered, complete and passed range and consistency checks before the system would issue a treatment allocation
2. For the first 282 patients, glucose was not recorded. After patient 282, it was collected at randomisation. Glucose therefore was not available for those 282 patients
3. Risk predicted by 'novel' model of Konig et al (2008). This model predicts outcome at three months, but if we assume that those who die between three and six months were dependent at three months, and those who do not die between three and six months do not change their dependency status, then the risk estimates are likely to be quite accurate for death or dependency at six months.
4. Stroke clinical syndrome derived from baseline clinical features assigned by an algorithm (algorithm available on request).
5. Expert panel's blinded assessment of pre-randomisation scan. This assessment was performed by the expert panel members after randomisation & blinded to treatment allocation and all clinical details.
6. Indeterminate because no infarct was visible.
7. Heparin: unfractionated or low-molecular weight heparin
8. Pre-trial experience of thrombolysis is defined as having had a protocol for open label rtPA and had treated at least 3 people in the 12 months before joining the trial; 76 (49%) centres met this criterion.

Table 2 Outcome at six months

					Adjusted		Unadjusted		
	No.	**(%)[1]**	**No.**	**(%)[1]**	**OR[2] (95% CI)**	**p**	**OR (95% CI)**	**P[3]**	**Events prevented per 1000 (SE)**
No. randomised									
a) Missing vital and disability status		.				.			.
b) Known to be alive, imputed 6 month disability status		.				.			.
c) Disability status at 6 months known									
d) No. with known or imputed data for analysis (b+c)									
Oxford Handicap Score									
0									.
1									.
2									.
3									.
4									.
5									.
6 (Died before six months)									
Alive and favourable outcome (0+1)									
Dead or dependent (3+4+5+6)									

OR = Odds Ratio. Notes: 1 Percentages of the totals with known or imputed six month data. 2. Odds ratio and p value calculated from logistic regression after adjusting for age (linear), NIHSS (linear), time (linear) and presence/absence of visible acute ischaemic change on baseline scan. 3 Significance probability calculated from test of difference between percentages for rt-PA and Control, using normal approximation. Oxford Handicap Scale: 0. no symptoms at all. 1. symptoms but these do not interfere with everyday life. 2. symptoms which have caused some changes in lifestyle but still able to look after oneself. 3. symptoms which have significantly changed lifestyle and I need some help in looking after myself. 4. severe symptoms requiring help from other people but I am not so bad as to need attention day and night. 5. severe handicap needing constant attention day and night

Table 3 Fatal and non-fatal cerebral events within first seven days of randomisation

	rt-PA		Placebo		Adjusted			Unadjusted				
	No.	(%)[1]	No.	(%)[1]	OR	95% CI	p	OR	95% CI	p	Events prevented per 1000 (SE)	
Number with seven day data												
CEREBRAL EVENTS[2]												
Neurological deterioration due to swelling of original infarct												
Non-fatal[3]												
Fatal												
Total												
Recurrent ischaemic stroke												
Non-fatal												
Fatal												
Total												
Recurrent stroke of unknown type												
Non-fatal												
Fatal												
Total												
Symptomatic intracranial haemorrhage[3]												
Non-fatal												
Fatal												
Total												
Neurological deterioration not due to swelling or symptomatic intracranial haemorrhage												
Non-fatal												
Fatal												
Total												
NON-CEREBRAL EVENTS												
Myocardial infarction												
Non-fatal												
Fatal												
Total												

	rt-PA		Placebo		Adjusted			Unadjusted				
	No.	(%)[1]	No.	(%)[1]	OR	95% CI	p	OR	95% CI	p	Events prevented per 1000 (SE)	
Major extracranial haemorrhage												
Non-fatal												
Fatal												
Total												
Allergic reaction												
Non-fatal												
Fatal												
Total												
Total deaths from cerebral causes < 7 days												
Total deaths from non-cerebral causes < 7 days												
Total deaths from any cause < 7 days												

1 Percentages of the totals with seven day data are shown. 2. The adjudication committee reviewed all cerebral events and deaths within 7 days. 3. Non fatal is defined as: event onset within 7 days, patient alive at day 7.3. The IST3 definition of symptomatic fatal or non-fatal intracranial haemorrhage is closely similar to that in ECASS-3, i.e. that the haemorrhage should be associated with clinically significant deterioration or death within 7 days, and the neuroradiological assessment of the imaging performed after the deterioration was that the haemorrhage contributed significantly to the deterioration. The frequency of symptomatic haemorrhages according to definitions used in earlier trials will be reported in subsequent publications.

Table 4 **Interaction of rt-PA treatment effect on primary outcome with age, sex, initial stroke severity, predicted risk, time to randomisation and stroke syndrome**

Subgroup	Events / Patients		Odds Ratio[1]	(95% C.I.)	p value for Interaction[2]
	rt-PA	Control			
***Age**					
Up to 80 years					
81 years and over					
***NIH Stroke score**					
0 - 5					
6 - 15					
16 - 24					
>=25					
***Predicted probability of poor outcome at 6 months [3]**					
< 0.4					
0.4 – 0.5					
0.5 – 0.75					
> 0.75					
***Time to randomisation**					
0-3 hours					
3-4.5 hours					
>4.5 hours					
Sex					
Female					
Male					
Stroke syndrome					
TACI					
PACI					
LACI					
POCI					
Clinician's assessment of scan at randomisation					
No evidence recent ischaemic change					
Possible evidence of recent ischaemic change					
Definite evidence of recent ischaemic change					
***Features of randomisation scan according to expert panel[1]**					
Acute ischaemic change					
No					
Yes					

1. Odds of being dead or dependent at six months for rt-PA group divided by odds for Control group.
2. Where a factor has more than two levels the test is for the null hypothesis that all levels have the same underlying odds ratio versus the alternative that the odds ratios have a linear trend (if the levels are ordered), or simply that the odds ratios are not all equal (if the levels are not ordered).
3. Probability predicted by model of Konig et al (2008). This model predicts outcome at three months, but if we assume that those who die between three and six months were dependent at three months, and those who do not die between three and six months do not change their dependency status, then the risk estimates are likely to be quite accurate for death or dependency at six months.
*key pre-specified subgroups

Webtable 1 Compliance with trial treatment protocol

		rt-PA		Control	
		No.	(%)	No.	(%)
Randomisation violations[1]					
	Dependent pre-stroke[2]				
	Haemorrhage on pre-randomisation scan				
	Advanced ischaemic change on pre-randomisation scan[3]				
	Tumour or non-stroke lesion on pre-randomisation CT[4]				
	Pre-randomisation low dose heparin				
	Pre-randomisation full dose heparin[5]				
	Systolic BP<90 or >220mmHg or diastolic BP<40 or >130mmHg				
	Glucose outside allowable limits (3.0 to 20 mmol/l)				
	Thrombolysis for stroke within previous 14 days				
Infusion compliance[6]					
	No drug given				
	Bolus only given				
	Infusion only given				
	Incomplete infusion				
	Wrong dose given[7]				
	Treated at >6 hours post stroke				
Treatments given within 24 h					
Double blind phase	Aspirin given				
	Other antiplatelet given				
	No antiplatelet given				
	Low dose heparin for DVT prophylaxis given				
	Full dose heparin given				
Open phase[8]	Aspirin given				
	Other antiplatelet given				
	No antiplatelet given				
	Low dose heparin for DVT prophylaxis given				
	Full dose heparin given				
	Intravenous fluids				
	Insulin				
Other treatments given between 24h and 7 days					
	Aspirin				
	Other antiplatelet				
	Low dose heparin or LMWH for DVT prophylaxis				
	Full anti-coagulation[9]				
	Any treatment to lower blood pressure				
	Antibiotics				
	Feeding via nasogastric tube or percutaneous gastrostomy				
Place of treatment in 7 days since randomisation (no. days and median)					
	Admissions area[10]				
	Stroke unit or stroke rehabilitation unit				
	High dependency ward, intensive care ward or critical care area				
	General ward[11]				

1. Base of percentages is number with scan data, randomisation record or seven day follow-up record in given treatment group. Data throughout this table are based on Yes/No questions. Numerators are numbers who responded Yes; denominators include both 'No' and 'Missing', so long as a form with some data was returned. All patients with a protocol violation were retained in the analysis.

2. In the early part of the trial, patients with a minimal degree of dependency could be included. After a protocol amendment to change eligibility, the randomisation programme was changed in September 2004 and such patients could not be included in the remainder of the trial. See main text.
3. Marked degree of ischaemic change on pre-randomisation CT or MR incompatible with onset less than 6 hours previously.
4. Tumour or non-stroke lesion sufficient to account for symptoms leading to randomisation.
5. Full-dose unfractionated heparin or high dose low molecular weight heparin
6. Base of percentages is number with valid infusion record in given trial phase and treatment group.
7. Dose violations occur when dose given is greater than 10% above or below the prescribed dose, or when a Control patient in the Open phase received any dose of rt-PA.
8. Patients in the control arm of the open phase who receive these drugs are not protocol violators, but are shown here for information. Base of percentages is number with valid seven day follow-up in given trial phase and treatment group.
9. Full-dose unfractionated heparin, high dose low molecular weight heparin or oral anticoagulants
10. Accident and Emergency Department or Medical admissions unit
11. General Ward: Neurology Ward, Geriatric Medicine Ward, General Internal Medicine Ward, Neurosurgical Ward, Geriatric Ward, Rehabilitation Ward or Other Ward.

Webtable 2: analysis restricted to patients with known disability status at six months

	Adjusted analysis						Unadjusted		
	No.	**(%)[1]**	**No.**	**(%)[1]**	**OR[2] (95% CI)**	**p**	**OR (95% CI)**	P[3]	**Events prevented per 1000 (SE)**
No. randomised									
Oxford Handicap Score									
0									.
1									.
2									.
3									.
4									.
5									.
6 (Died before six months)									
Alive and favourable outcome (0+1)									
Dead or dependent (3+4+5+6)									

OR = Odds Ratio. Notes: 1 Percentages of the totals with known or imputed six month data. 2. Odds ratio and p value calculated from logistic regression after adjusting for age (linear), NIHSS (linear), time (linear) and presence/absence of visible on baseline scan. 3 Significance probability calculated from test of difference between percentages for rt-PA and Control, using normal approximation. Oxford Handicap Scale: 0. no symptoms at all. 1. symptoms but these do not interfere with everyday life. 2. symptoms which have caused some changes in lifestyle but still able to look after oneself. 3. symptoms which have significantly changed lifestyle and I need some help in looking after myself. 4. severe symptoms requiring help from other people but I am not so bad as to need attention day and night. 5. severe handicap needing constant attention day and night

Figure 1

CONSORT 2010 Flow Diagram

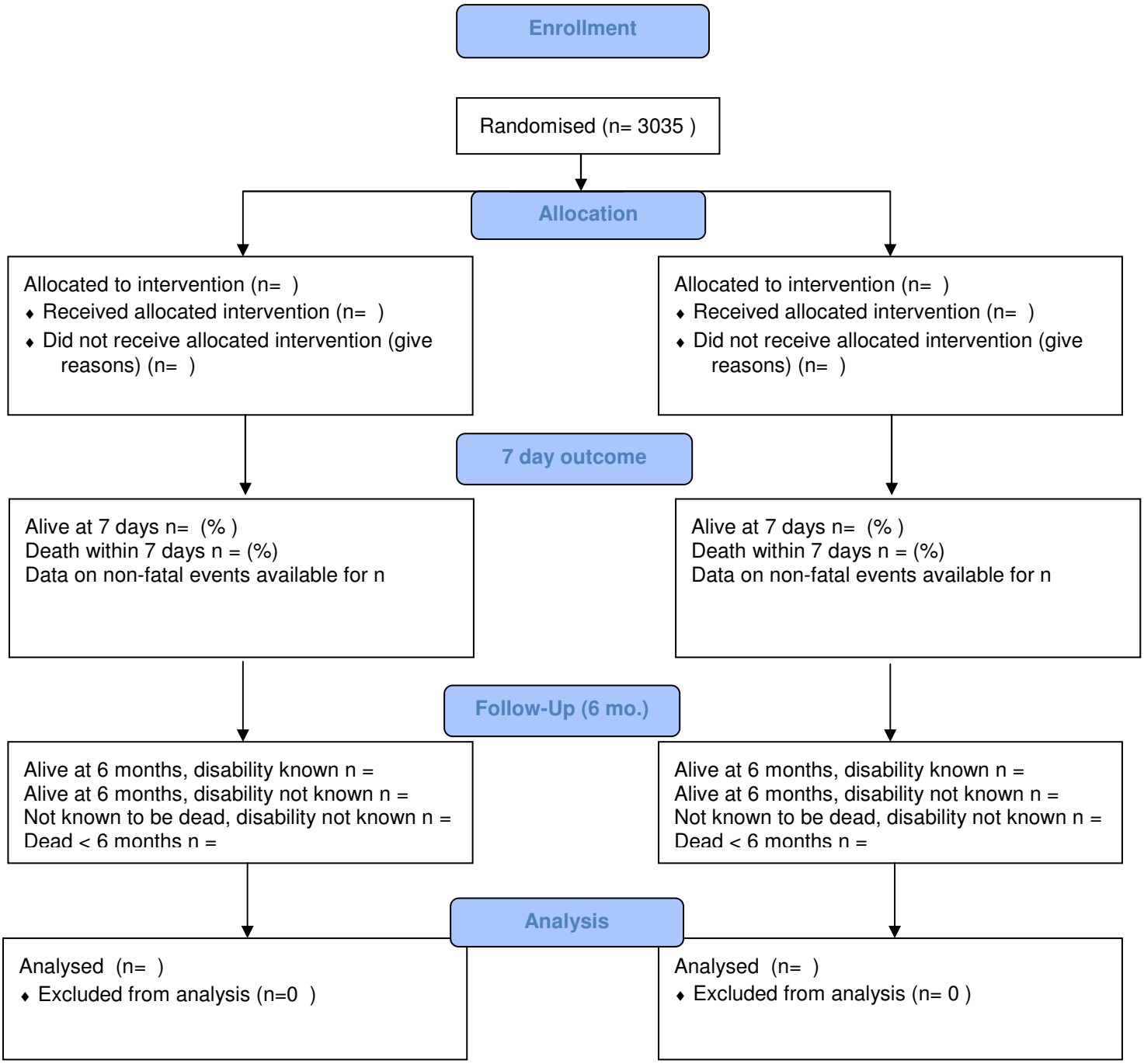
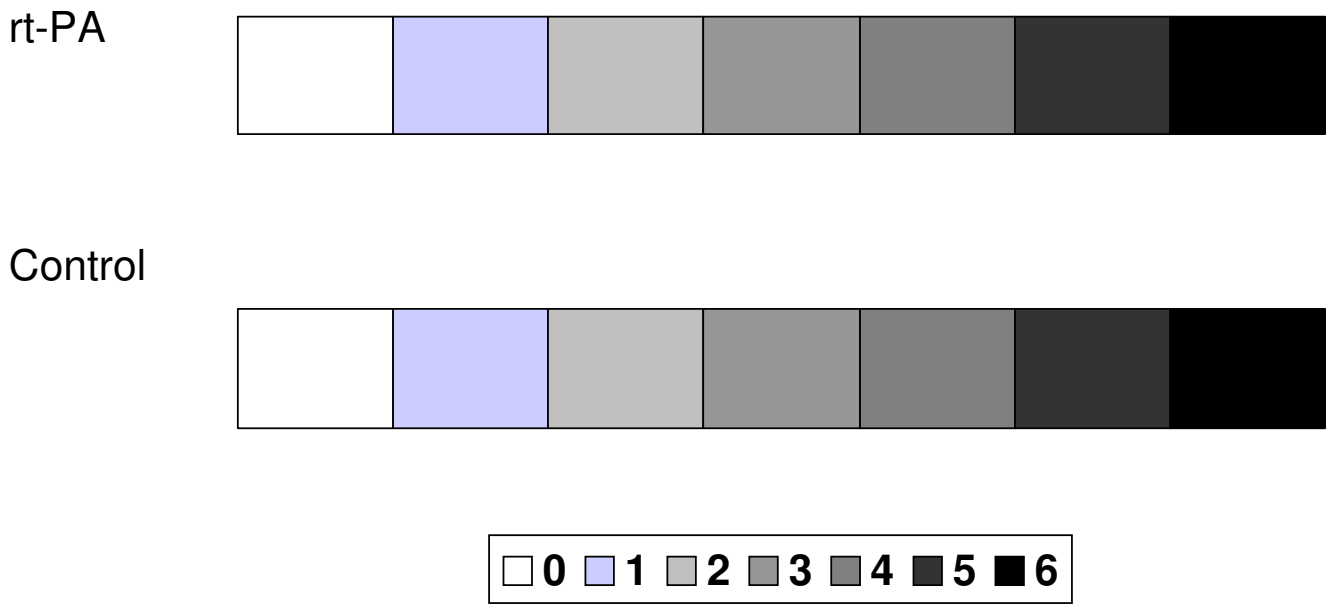


Figure 2 Bar chart of OHS in each treatment group (created with powerpoint)



Bar chart displaying each OHS grade in each treatment group

Figure 3

Forest plot of treatment effect on the primary outcome among different subgroups. P value is the p value for a test of interaction

Figure 4 (may be omitted if updated Cochrane review published simultaneously)

Forest plot. Systematic review of all trials of rt-PA for acute ischaemic stroke: effect on primary outcome

Figure 5

Kaplan-Meier survival analysis rt-PA vs control

Appendix 1

Proposed content of primary and subsequent publications

2012

- Main results paper 1: effects on events within seven days and primary outcome at six months and pre-specified subgroup analyses on primary outcome and main pre-specified risks. Tables 1-5, webtable 1,2 & figures 1,2,3,4,
- Main results paper 2: IST3 results in context of updated Cochrane systematic review of RCT's of thrombolysis for stroke
- Further subgroup analyses of primary outcome plus exploratory analyses of secondary outcomes (Tables in Appendix 3 indicate possible content).
- Imaging publications to include
  - Structural imaging descriptive analyses
  - Correlation between perfusion imaging and attenuation changes on plain CT
  - Features on baseline scan, which, taken in conjunction with clinical variables that modify response to treatment (measured both by effects on the primary outcome but also on the key secondary outcomes of intracranial bleeding and infarct swelling); with a particular emphasis on identifying which patients are likely to develop SICH with rt-PA.
  - Hyperdense artery sign appearance/disappearance and general value
  - Clinical - radiological correlations (eg baseline imaging with OCSP subtype)

2013 and subsequent possible analyses and publications

- Imaging analyses
  - Methods for processing perfusion data
  - Value of pre-randomisation MR, MRP, DWI, CTA CTP etc
- Secondary clinical outcomes: HRQOL, longer-term survival, with exploratory subgroups on early and late outcomes
- Stroke Thrombolysis Trialists Collaboration (STTC) Individual patient data meta-analysis of all i.v. rt-PA RCT's to determine which patients to treat, which not.
- Prognostic models for acute stroke (W Whitely)
- Health Economic analyses: updating our HTA economic model and re-estimating cost effectiveness (in collaboration with Eivind Berge and Veronica Murray)
- Relation between change in blood pressure in acute ischaemic stroke and risk of early adverse events and poor long-term outcome. (E Berge and L Sandset)
- Effect on treatment on placement at 6 months (non-medical cost to society for the consequences of stroke related disability)
- Do the resources invested in the adjudication process in IST-3 yield greater precision in the trial result (compared with an analysis based on unadjudicated data)?
- Collaborative meta- analysis with NINDS group to examine impact of thrombolysis on long-term survival after stroke (and assess impact of level of disability at 6 months on subsequent survival
- Methods of attaining 6 month follow up - postal versus telephone, the baseline predictors of whether a patient responds to post, and validation of any models we produce in CLOTS 1and 2.

Appendix 2

Definitions used by IST-3 adjudication committee for events within 7 days

A. If the patient was dead by day 7, a 'status at 7 days code' was assigned:

1. Death from initial stroke within 7 days of randomisation, attributed to infarct swelling.
There should be evidence of significant brain swelling on a post-randomisation scan (or autopsy if not re-scanned before death). This corresponds to either a response on Question Q16 of the Expert CT Readers Form (ECTRF) 'Shift of the midline away from the side of the ventricle or 'Effacement of the basal cisterns' **OR** a response to Question 5 of 'Midline shift' or 'Uncal herniation'. The presence of some degree of haemorrhagic transformation is permitted, provided it is not considered by the expert CT reader to be a major contributor to the mass effect.

2. Death from initial stroke within 7 days of randomisation, attributed to intracranial haemorrhage.
There should be clear evidence of significant intracranial haemorrhage on the post-randomisation scan (or autopsy if not re-scanned before death). Significant haemorrhage is present on any post-randomisation scan if the expert reader gives any response to Question 22 other than a blank value or 'Petechial haemorrhage' (i.e. significant HTI, parenchymal haematoma, etc) **AND** a response to Question 23 of 'yes', indicating that haemorrhage is a major component of the lesion (or is remote from the lesion and likely to have contributed significantly to the burden of brain damage). This event includes deaths attributed to a clinical event of recurrent stroke within 7 days, in which the recurrent stroke was confirmed to be due to an intracranial
haemorrhage.

3. Death from initial stroke within 7 days of randomisation, not definitely attributable either to infarct swelling or haemorrhage.
A post randomisation scan may show a large infarct with some degree of swelling, but swelling was coded in response to Question 5 as 'sulcal effacement', 'ventricular effacement' or 'sulcal effacement + ventricular effacement' **AND** response to Question 16 as 'None', 'Effacement of the sulci overlying the infarct', 'Minor effacement of the adjacent lateral ventricle', 'Complete effacement of the lateral ventricle', or 'Effacement of the lateral and third ventricle' **AND** no significant haemorrhage was present. If the initial stroke was severe, include deaths within 7 days from pneumonia, and deaths within 7 days with no additional information available.

4. Death due to recurrent ischaemic stroke within 7 days.
There should be clear clinical evidence of recurrent stroke, and no evidence of significant haemorrhage on the post-randomisation scan (or autopsy if not rescanned before death).

5. Death due to recurrent stroke of unknown type within 7 days.
Death should only be assigned to this category if there was clear clinical evidence of recurrent stroke, and no scan was performed after the recurrence and no autopsy was performed.

6. Death due to non-cerebral causes.
If the clinician completing the 7 day form attributes the death to a non neurological cause (extracranial haemorrhage, ischaemic heart disease, pulmonary embolism, other vascular cause, or a non-vascular cause) the assigned cause will be employed in the main analyses.

B. If the patient was alive at day 7, one of the following 'status at 7 days code' codes was assigned:

7. Neurological deterioration within 7 days of randomisation, attributed to swelling of the initial ischaemic stroke.
There should be evidence of significant brain swelling on the post-randomisation scan (or autopsy if not re-scanned within 7 days and death occurs after 7 days). There should be evidence of significant brain swelling on a post-randomisation scan (or autopsy if not re-scanned before death). This corresponds to either a response on Question 16 of the Expert CT Readers Form (ECTRF) 'Shift of the midline away from the side of the ventricle or 'Effacement of the basal cisterns' **OR** a response to Question 5 of 'Midline shift' or 'Uncal herniation'. The presence of some degree of haemorrhagic transformation is permitted, provided it is not considered by the expert CT reader to be a major contributor to the mass effect.

.

8. Symptomatic intracranial haemorrhage within 7 days of randomisation. There should be clear evidence of significant intracranial haemorrhage on the post-randomisation scan (or autopsy if not re-scanned and death occurs after 7 days). Significant haemorrhage is present on any post-randomisation scan if the expert reader gives any response to Question 22 other than a blank value or 'Petechial haemorrhage' (i.e. significant HTI, parenchymal haematoma, etc) **AND** a response to Question 23 of 'yes', indicating that haemorrhage is a major component of the

lesion (or is remote from the lesion and likely to have contributed significantly to the burden of brain damage). This event includes clinical events described as a recurrent stroke within 7 days, in which the recurrent stroke was confirmed to be due to an intracranial haemorrhage.

9. Neurological deterioration within 7 days of randomisation, not attributable to brain swelling.
A post randomisation scan may show a large infarct with some degree of swelling, but swelling was coded in response to Question 5 as 'sulcal effacement', 'ventricular effacement' or 'sulcal effacement + ventricular effacement' **AND** response to Question 16 as 'None', 'Effacement of the sulci overlying the infarct', 'Minor effacement of the adjacent lateral ventricle', 'Complete effacement of the lateral ventricle', or 'Effacement of the lateral and third ventricle' **AND** no significant haemorrhage was present.

10. Recurrent ischaemic stroke within 7 days.
There should be clear clinical evidence of recurrent stroke, and no evidence of significant haemorrhage on the post-randomisation scan (or autopsy if not re-scanned and death occurs after 7 days).

11. Recurrent stroke of unknown type within 7 days.
Clear clinical evidence of recurrent stroke, but no post-randomisation scan or autopsy was performed.

Appendix 3

1. Proposed format of some tables to be included in subsequent publications

Secondary outcomes at six months

	rt-PA		Control	
	No.	(%)[1]	No.	(%)[1]
No. randomised		.		
Total deaths before six months				
Stroke left patient with problems				
No				
Yes				
Died				
Patient needs help with everyday activities				
No				
Yes				
Died				
Patient needs help to walk				
No				
Yes				
Died				
Patient has major problems with speaking				
No				
Yes				
Died				
EuroQol score (≤ 0=worst, 100=best)				
81-100				
61-80				
41-60				
21-40				
0-20				
Negative value				
Dead				
Patient's current residence				
In own home alone				
In own home with partner or relative				
In home of a relative				
In a residential home				
In a nursing home				
In hospital				
Dead				

[1] Percentages of the totals with six month data will be shown

2. Interaction of rt-PA treatment effect on primary outcome with initial and expert readings of pre-randomisation brain scan

Subgroup	Events / Patients		Odds	(95%	p value for
	rt-PA	Control	Ratio[1]	C.I.)	Interaction[2]
Infarct on scan according to clinician's opinion at randomisation					
Definite					
Possible					
No					
Size of tissue lesion					
None					
Small					
Medium					
Large					
Very large					
Depth of tissue damage					
None					
Mild					
Severe					
Degree of swelling					
None					
Sulcal					
Ventricular					
Hyperdense Artery					
None					
Anterior					
Posterior					
Atrophy					
No					
Yes					
Periventricular lucencies					
No					
Yes					
Old vascular lesion					
No					
Yes					

1. Odds of being alive and independent at six months for rt-PA group divided by odds for Control group.
2. Where a factor has more than two levels the test is for the null hypothesis that all levels have the same underlying odds ratio versus the alternative that the odds ratios have a linear trend (if the levels are ordered), or simply that the odds ratios are not all equal (if the levels are not ordered).

3. **Interaction of rt-PA treatment effect on primary outcome with trial phase, centre experience, antiplatelet treatment pre-randomisation, atrial fibrillation and blood pressure at randomisation**

Subgroup	Events / Patients		Odds Ratio[1]	(95% C.I.)	p value for Interaction[2]
	rt-PA	Control			
Trial phase					
Double blind					
Open					
Centre with thrombolytic experience for acute stroke					
Yes					
No					
Antiplatelets given < 48 hours before randomisation					
Yes					
No					
Atrial fibrillation					
Yes					
No					
Systolic blood pressure at randomisation (mm Hg)					
<= 144					
145 to 164					
>= 165					
Diastolic blood pressure at randomisation (mm Hg)					
<= 74					
75 to 89					
>= 90					

1. Odds of being alive and independent at six months for rt-PA group divided by odds for Control group.
2. Where a factor has more than two levels the test is for the null hypothesis that all levels have the same underlying odds ratio versus the alternative that the odds ratios have a linear trend (if the levels are ordered), or simply that the odds ratios are not all equal (if the levels are not ordered).

Appendix 4

Oxford Handicap Scale postal questionnaire

Tick ✓ONE box next to the sentence which best describes your present state.

- ☐ I have no symptoms at all
- ☐ I have a few symptoms but these do not interfere with my everyday life
- ☐ I have symptoms which have caused some changes in my life but I am still able to look after myself
- ☐ I have symptoms which have significantly changed my life and I need some help in looking after myself
- ☐ I have quite severe symptoms which mean I need to have help from other people but I am not so bad as to need attention day and night
- ☐ I have major symptoms which severely handicap me and I need constant attention day and night

Supplementary questions

Please tick ✓one box on each line	**YES**	**NO**
Has the stroke left you with any problems?	☐	☐
Do you need help from anybody with everyday activities? (in washing, dressing, feeding & toileting)	☐	☐
Do you need help from anybody to walk?	☐	☐
Do you have major problems with speaking?	☐	☐

Appendix 5

Contribution of the authors

The authors listed on the front page drafted this statistical analysis plan and commented on all drafts. It was prepared without knowledge of the unblinded data. The unblinded study statistician prepared tabulations of the baseline characteristics (for both treatment groups combined) of the patients recruited in the trial; the authors used these to inform their choice of cutpoints to define subgroups and some aspects of the overall analysis plan. The unblinded study statistician had no role in the choice of pre-specified subgroups for analysis. The plan was written independently of the sponsors, funding agencies for the trial and of Boehringer Ingelheim (the company which had provided the drug and matching placebo for the first phase of the trial).

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