IRIS Collaboration pooled analysis: Statistical Analysis Plan

Six trials have investigated direct endovascular treatment (EVT) has a superior or a non-inferior effect on functional outcome compared to EVT preceded by intravenous alteplase administration (IVT) for patients with acute ischemic stroke caused by an intracranial proximal large vessel occlusion.
The current six trials are:

- DEVT (n=234)
- DIRECT-MT (n=656)
- DIRECT-SAFE (n=295)
- MR CLEAN-NO IV (n=539)
- SKIP (n=204)
- SWIFT-DIRECT (n=408)

DIRECT-MT is completed and included 656 patients. It has a similar set-up to the MR CLEAN-NO IV trial. The study protocol demands that stent-retrievers should be used as 1st line of treatment strategy during the thrombectomy procedure. The trial had a non-inferiority design. Results were presented at ESO-WSO 2020 and published in NEJM 2020; 382:1981-93.
Trial registration number: NCT03469206 (https://www.clinicaltrials.gov/)
SKIP is completed and enrolled 204 patients, randomized for direct EVT or 0.6 mg/kg alteplase followed by EVT. EVT could be performed with a MERCI device, Penumbra, Trevo, Solitaire, Revive, any stents and percutaneous balloon angioplasty. Patients had to be <86 years old, have an initial NIHSS ≥ 6 and an initial ASPECTS ≥ 6 on CT or ≥ 5 on DWI. Patients with an ICA or M1 occlusion were eligible. The trial had a non-inferiority design. Results were presented on February 21, at ISC 2020 in LA and published in JAMA 2021:325(3): 244-253
Trial registration number: UMIN000021488 (https://www.umin.ac.jp/ctr/index.htm)
MR CLEAN-NO IV is completed and enrolled 539 patients randomized for EVT with or without pretreatment with 0.9 mg/kg alteplase. The protocol demanded that initially only CE- or FDA-approved stent-retrievers should be used as 1st line of treatment strategy during the thrombectomy procedure. The trial had a superiority design, but assessed non-inferiority as a secondary aim.
Results were presented at ISC 2021, March 18 and published in NEJM 2021 Nov 11;385(20):1833-1844. Trial registration number: ISRCTN80619088 (http://www.isrctn.com/)
DEVT followed a five-look group-sequential non-inferiority design, is completed and included 234 patients. Patients were randomized between direct EVT or bridging intravenous alteplase (0.9 mg/kg) followed by EVT in a 1:1 ratio. Inclusion criteria include ICA or M1 occlusion, randomization within 4h 15m of stroke onset. The trial had a non-inferiority design. Results were published in JAMA 2021; 325(3):234-243.
Trial registration number: ChiCTR-IOR-17013568 (http://www.chictr.org.cn/)
SWIFT-DIRECT is completed and enrolled 408 patients, randomized to EVT with or without pretreatment with 0.9 mg/kg alteplase. EVT could only be performed with stent-retriever revascularization devices manufactured by Medtronic (e.g. Solitaire). Patients had to have an initial NIHSS ≥ 8 and < 30, should be <86 years old, and EVT had to be deemed technically feasible. Patients with an intracranial ICA or M1 occlusion were eligible. The trial had a non-inferiority design. Results were presented at ESOC 2021 and were published in the

Lancet 2022; 400(10346):104-115.
Trial registration number: NCT03192332 (https://www.clinicaltrials.gov/)
DIRECT-SAFE has stopped enrollment and included 293 patients, also adhering to a dose of 0.9 mg/kg alteplase. Use of the Trevo stent retriever as first line of defense was mandated. Patients with an ICA, M1, M2 or basilar artery occlusion were eligible. The trial had a non-inferiority design. Results have been presented at ESOC 2021 and were published in the Lancet 2022; 400(10346):116-125.
Trial registration number: NCT03494920 (https://www.clinicaltrials.gov/)

The IRIS acronym represents all six trials, totaling 2334 patients. The pooling process will follow a sequential approach: after the first pooled analysis of completed trials (Preliminary starting date: January 2022), subsequently finished trials may be added to the pooled database. Although the current pooled analysis protocol only foresees the inclusion of the aforementioned trials, in the future additional similar trials may be added at the discretion of the Executive Committee (in which the current trials are represented).

1 IRIS Executive Committee oversight

The IRIS Executive Committee shall be jointly composed of two PI representatives from each of the 6 trials, including a maximum of 2 statisticians. Meetings will take place in person where appropriate and by teleconference as needed. See the Governance, Publication and Ancillary Studies policy. Prior to final analyses, the steering committee shall sign off on the SAP and it will be executed as planned.

2 Objective of the study

The aims of the study are

1) To provide a pooled analysis of individual patient data from these 6 trials, to investigate whether direct EVT is non-inferior to EVT with prior IVT in patients with an anterior circulation ischemic stroke due to a large vessel occlusion, by pre-specified non-inferiority margins.
2) To explore whether direct EVT is non-inferior to EVT with prior IVT by prespecified subgroups
3) To further investigate workflow implications of the results from aims 1) and 2)
4) To further investigate implications of imaging results from aims 1) and 2) (i.e., improving recanalization and reperfusion, thrombus imaging characteristics, etc.)
5) To improve patient selection and to contribute towards more tailored decision-making concerning IVT use in patients requiring EVT for acute ischemic stroke.

3 Methodology

3.1 Design

One step individual patient data meta-analysis of prospective, randomized, open-label trials with blinded outcome evaluation (PROBE) designs.

3.2 Planned analyses

The pooled analysis aim is to answer the main research question:
“Is direct EVT non-inferior to IVT followed by EVT in patients with anterior circulation large vessel occlusion acute ischemic stroke?”

Secondary analyses have been planned to take place after the main analysis is completed. All of these analyses will be considered confirmatory to existing RCT data or exploratory because power will be variable depending on the hypothesis.

1. Main Pooled Analysis paper, including key subgroups (see below).
2. Time metrics: the effect of direct EVT vs IVT+EVT on functional outcome modified over onset-to-groin puncture and other clinically meaningful time intervals.
3. Occlusion location: The effect of direct EVT vs IVT+EVT on functional outcome modified over and/or stratified by baseline CTA occlusion location.
4. Etiology: the effect of direct EVT vs IVT+EVT on functional outcome modified over stroke etiology (as collected by each individual trial).
5. Ethnicity: the effect of direct EVT vs IVT+EVT on functional outcome modified over and/or stratified by ethnicity (based on country of trial execution).
6. First-pass effect: association with IVT+EVT compared to direct EVT, association with 90-day clinical outcome, characteristics of patients who are more likely to have reperfusion prior to EVT.
7. Prediction model: building of prediction model (e.g., by means of regression models or machine learning) to predict patient benefit of IVT prior to EVT

3.2 Data preparation and access

3.2.1 Methodology of pooling data

A list of core variables was determined based on the pooled available data from the collaborating trials and pre-specified required variables.
No data will be pooled until each trial's steering committee has agreed to release the data.
All data and reports from these data shall be the property of the 6 trials and the statistician jointly.
For the primary papers all data will in principle be used as is, i.e. no data will be rescored and no clinical data will be re-assessed or newly collected. For secondary papers new data can be collected and these data can be merged with the database at a later date.
Only variables with sufficient homogeneity and completeness will be included in the final dataset and analysis.

3.2.2 Imaging data

The data extracted from imaging will be managed by the Data and Imaging Committee and coordinators of the individual trials. The goal will be to provide a harmonized interpretation of all imaging data. Data extracted from the imaging by each trial's core-labs will be centrally collected; the imaging itself will not be centrally collected. Imaging Case Report Forms from each trial should be comparable and consensus on definitions should be reached regarding data on the following topics, where possible with regard to data quality and availability, as summarized and collected in the Data Dictionaries.

As stated above, for the primary papers the data will be used as is, and only if data is homogeneous enough to be used. For further papers potential re-assessment and harmonization is possible. However, for this the trials in charge of the relevant substudies are in charge and should organize the process after consulting the Data & Imaging committee.

3.2.2 Data curation and access

The data will be held and curated by an independent statistician (prof. Hester Lingsma), supported by the supportive statistician where needed (prof. Leonid Churilov) independent of the sponsor and each of the trial investigator groups.
The dataset will be made available through an online digital workspace (anDREa) to participating trial teams. This ensures all teams can run their own analysis while the data remains secure, preventing data leaks. Moreover, this allows training and involvement of more junior team members in data analysis.

3.3 Definition of the target populations

3.3.1 Efficacy population

The current study features a modified intention to treat population. All patients randomized for direct EVT or EVT + IVT with an anterior circulation acute ischemic stroke from the 6 pooled trials will be included if they provided consent. Protocol violation cases will not be excluded if they had been randomized and reported on the original pooled trials.

3.2.1.1 Efficacy population sample size

The population sample size will be determined by the total population that fulfills the efficacy population definition. No statistical power calculations will be performed.

3.3.2 Sensitivity analyses

Two proposed analyses will be performed;
1. A separate modified intention-to-treat analysis in a population including patients from trials with a pre-specified dose of 0.9mg/kg. Effectively this will exclude patients from the SKIP trial.
2. An as-treated analysis with the following patients:
    a. All patients allocated to IVT+EVT who received intravenous alteplase or tenecteplase. Patients randomized to IVT+EVT in whom successful reperfusion was achieved before completion of alteplase infusion, in whom the infusion was subsequently stopped are an exception. These patients are also included in the as-treated analysis as this might reflect future clinical practice. Any patients for whom alteplase was started after groin puncture are excluded.
    b. All patients allocated to direct EVT who did not receive any intravenous alteplase or Tenecteplase prior to start of EVT. Patients who were randomized to direct EVT and who received intravenous alteplase or Tenecteplase after EVT because of incomplete reperfusion, are included in the as-treated analysis, since administration of IVT after failed EVT was part of the strategy of direct EVT in some trials. Exclusion of these patients would also bias the analysis in favor of direct EVT.
3. A separate as-treated analysis as described above, including patients from trials with a pre-specified dose of alteplase 0.9mg/kg. Effectively this will exclude patients from the SKIP trial and patients who received a different thrombolytic (i.e. urokinase or tenecteplase).

3.4 Pooled Analysis: outcomes

3.4.1 Primary outcome:

- Ordinal mRS at 90 days

3.4.2 Secondary outcomes:

- mRS dichotomizations (mRS 0-2 indicating functional independence; mRS0-1; mRS 0-3)

- NIHSS at 3-7 days (or discharge if earlier)
- Early reperfusion defined as absence of treatable occlusion or eTICI ≥2B on first DSA of the proximal occlusion on baseline CTA occlusion.
- Final reperfusion on eTICI scale
    - Reperfusion ≥2B, reperfusion ≥2C

3.4.3 Safety outcomes:

- Intracranial hemorrhage (sICH according to Heidelberg Bleeding Criteria, any ICH)
- Mortality at 90 days

3.6 Main paper statistical analysis

3.6.1 Primary analysis

The primary analysis will assess non-inferiority of direct EVT compared to IVT + EVT conducted on a modified intention-to-treat basis using a mixed effect ordinal regression model with the 90-day modified Rankin Scale score as outcome. The primary outcome measure is the adjusted common Odds Ratio for a shift in the direction of better outcome on the modified Rankin Scale.
Mixed effect modelling will be used to account for between trial differences in treatment effect.

<u>All analyses will be adjusted for the following prognostic variables:</u>

- Age
- ASPECTS
- Atrial fibrillation
- Occlusion location on baseline CTA/MRA
- Baseline NIHSS
- Pre-stroke mRS score
- Time from onset to randomization

Both unadjusted and adjusted estimates are reported with corresponding 95%CI. The adjusted analysis will be used as primary outcome.

3.6.2 Non-inferiority margin

For this analysis we assess non-inferiority of direct EVT compared to alteplase or Tenecteplase followed by EVT.
To define non-inferiority, a margin of 5% was determined to be the maximum clinically acceptable difference in the proportion of patients with good outcome (mRS 0-2) between the two treatment arms. This was done according to the expedited ESO-ESMINT guideline regarding intravenous alteplase treatment before EVT and was the most frequently chosen non-inferiority boundary for this specific question in a recent survey (Kaesmacher et al. JNIS 2022, doi: 10.1136/neurintsurg-2022-018665). Based on the pooled mRS 0-2 rate of all 6 RCT's direct EVT arms (49%), a 5% higher mRS 0-2 rate in favor of combined treatment (54%) would result in an OR of 0.82. To assess non-inferiority we determine whether the lower bound of the two-sided 95%CI of the adjusted common Odds Ratio (acOR) for a shift in the direction of better outcome on the mRS after direct EVT does not cross 0.82. Establishing non-inferiority using this boundary would translate into 97.5% confidence that the benefit of combined treatment would not exceed a 5% increase in functional

independence. For illustration purposes we will also derive the absolute risk difference of mRS 0-2 between the direct EVT and IVT + EVT arms corresponding to the estimated acOR and its 95% confidence interval for an average patient.

3.6.3 Secondary and safety outcomes

For dichotomous outcomes, mixed effects binary logistic regression models will be used to determine an odds ratio (adjusted and unadjusted).
For continuous outcomes, regression beta coefficients as determined with mixed effect linear regression models will be determined. Outcomes will be transformed if necessary to adhere to the assumptions.

For the secondary outcomes and the safety outcomes, the same random effect terms and adjustment variables will be used as for the primary outcome analysis (paragraph 3.6.2). Both unadjusted and adjusted estimates will be reported with corresponding 95%CI.

3.6.4 Sensitivity analysis

Additional analyses will include the analyses in populations defined in paragraphs 3.3.2. Alternate methods may be considered and will be approved by general consensus of the Executive Committee and the independent statistician.

3.6.5 Handling of missing data

Under the ITT principle, all patients with anterior circulation strokes who are randomized are included in the analysis. Therefore, missing data, especially in the outcome measures, can be problematic. Every effort will be made to reduce the amount of missing data on the final database to a minimum. These include the use of less granular variable definitions and structures when needed to allow heterogeneously data collected by different approaches from the 6 trials to be used in a reasonable manner. In the event that, despite the clinical centers' best efforts, there are missing data, missing baseline and outcome data will be imputed for all performed regression analyses with multiple imputation methods provided by the MICE package for R. Outcome variables were selected based on data consistently available for every one of the 6 trials. For outcome variables imputation will only be conducted if the variable is considered to be reasonably complete for every individual trial.
All original baseline, intermediate outcome and final outcome data will be used for imputation predictions. Additionally, including study will be included as a variable in the imputation model. Variables identified as constant or perfectly colinear with other variables may be removed from the imputation prediction matrix. A total of 5 imputation iterations will be performed and pooled for analysis.
Only the variables needed as independent/dependent variables of interest or adjustment variables will actually be imputed.
If an assessment was conducted outside of the protocol-specified time window, data obtained are still included in the analysis, with the rationale that it is a more accurate measure than those obtained by imputation.
Imputed data will be used for statistical modeling and effect estimations. Imputed data will not be included in the descriptive statistics listed in baseline and outcome tables as well as descriptive figures.

3.6.6 Key subgroups

Although subgroups are defined in categories, the full (appropriately converted) scale or range should be used in analyses where possible, to avoid loss of information.

Subgroup analyses will be done for the primary outcome only, using the model described above resulting in an adjusted common OR with corresponding 95% CI. Unadjusted subgroup analysis results will be presented in the supplement; adjusted results in the manuscript main body. P-values for interactions and explorative effect estimates of the primary effect measure with corresponding 95% CI in subgroups will be presented. Treatment interaction will be tested in separate models. The interaction effect between the prespecified subgroups and the treatment arms will be dtermined in a two-step manner with pooling of estimates on a per study basis to mitigate the risk of bias. For each subgroup analysis p-values, estimates (adjusted OR) and 95% confidence interval will be reported in the main text and/or in a forest-plot table. No inference regarding non-inferiority will be made for subgroups.
The prespecified subgroup analyses are:

- Sex
- Age (subgroups 18-64 years, 65-79 years, 80 or older; interaction term calculated based on continuous variable)
- Time intervals: symptom onset to groin puncture and onset to randomization time (subgroups by tertiles; interaction term calculated based on continuous variable)
- Occlusion location (ICA-T, M1, M2)
- ASPECTS (subgroups 0-5, 6-10; interaction term calculated based on granular variable)
- Baseline NIHSS (subgroups by tertiles; interaction term calculated based on continuous variable)
- Tandem extracranial ICA lesion y/n
- Atrial fibrillation vs no atrial fibrillation

3.6.7 Secondary/Exploratory analyses

Multiple secondary analyses may be possible as described in the pooling proposal above. A publication policy will be circulated after approval of this document, including a request to develop new proposals. Any such (non-pre-specified) analyses, that arise after the pooled-analysis SAP is finalized, will be considered exploratory and stated as such. No adjustments for multiplicity will be made for these exploratory analyses. The exploratory analyses will be expected to follow the broad statistical methods guidelines as specified by the statistician(s) and approved by the Executive Committee.

All papers following the main paper are advised to follow the main paper's statistical methods, where possible. Supporting materials (e.g. example imputation code, presentation formats, patient selection flowchart model) will be made available to all substudy researchers.

Appendix: Collaboration name & Logo

IRIS: Improving Reperfusion strategies in Ischemic Stroke

Logo: