Extending thrombolysis to 4·5–9 h and wake-up stroke using perfusion imaging: a systematic review and meta-analysis of individual patient data

Bruce C V Campbell, Henry Ma*, Peter A Ringleb*, Mark W Parsons, Leonid Churilov, Martin Bendszus, Christopher R Levi, Chung Hsu, Timothy J Kleinig, Marc Fatar, Didier Leys, Carlos Molina, Tissa Wijeratne, Sami Curtze, Helen M Dewey, P Alan Barber, Kenneth S Butcher, Deidre A De Silva, Christopher F Bladin, Nawaf Yassi, Johannes A R Pfaff, Gagan Sharma, Andrew Bivard, Patricia M Desmond, Stefan Schwab, Peter D Schellinger, Bernard Yan, Peter J Mitchell, Joaquín Serena, Danilo Toni, Vincent Thijs, Werner Hacke†, Stephen M Davis†, Geoffrey A Donnan†, on behalf of the EXTEND, ECASS-4, and EPITHET Investigators‡*

Summary

Background Stroke thrombolysis with alteplase is currently recommended 0–4·5 h after stroke onset. We aimed to determine whether perfusion imaging can identify patients with salvageable brain tissue with symptoms 4·5 h or more from stroke onset or with symptoms on waking who might benefit from thrombolysis.

Methods In this systematic review and meta-analysis of individual patient data, we searched PubMed for randomised trials published in English between Jan 1, 2006, and March 1, 2019. We also reviewed the reference list of a previous systematic review of thrombolysis and searched ClinicalTrials.gov for interventional studies of ischaemic stroke. Studies of alteplase versus placebo in patients (aged ≥18 years) with ischaemic stroke treated more than 4·5 h after onset, or with wake-up stroke, who were imaged with perfusion-diffusion MRI or CT perfusion were eligible for inclusion. The primary outcome was excellent functional outcome (modified Rankin Scale [mRS] score 0–1) at 3 months, adjusted for baseline age and clinical severity. Safety outcomes were death and symptomatic intracerebral haemorrhage. We calculated odds ratios, adjusted for baseline age and National Institutes of Health Stroke Scale score, using mixed-effects logistic regression models. This study is registered with PROSPERO, number CRD42019128036.

Findings We identified three trials that met eligibility criteria: EXTEND, ECASS4-EXTEND, and EPITHET. Of the 414 patients included in the three trials, 213 (51%) were assigned to receive alteplase and 201 (49%) were assigned to receive placebo. Overall, 211 patients in the alteplase group and 199 patients in the placebo group had mRS assessment data at 3 months and thus were included in the analysis of the primary outcome. 76 (36%) of 211 patients in the alteplase group and 58 (29%) of 199 patients in the placebo group had achieved excellent functional outcome at 3 months (adjusted odds ratio [OR] 1·86, 95% CI 1·15–2·99, p=0·011). Symptomatic intracerebral haemorrhage was more common in the alteplase group than the placebo group (ten [5%] of 213 patients *vs* one [<1%] of 201 patients in the placebo group; adjusted OR 9·7, 95% CI 1·23–76·55, p=0·031). 29 (14%) of 213 patients in the alteplase group and 18 (9%) of 201 patients in the placebo group died (adjusted OR 1·55, 0·81–2·96, p=0·66).

Interpretation Patients with ischaemic stroke 4·5–9 h from stroke onset or wake-up stroke with salvageable brain tissue who were treated with alteplase achieved better functional outcomes than did patients given placebo. The rate of symptomatic intracerebral haemorrhage was higher with alteplase, but this increase did not negate the overall net benefit of thrombolysis.

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Introduction

Current guidelines recommend thrombolysis with intravenous alteplase for acute ischaemic stroke up to 4·5 h from stroke onset on the basis of individual patient data from randomised trials that included patients according to non-contrast CT brain and clinical criteria.[1] These criteria exclude a substantial proportion of patients who present more than 4·5 h after stroke onset or who have unknown onset (eg, wake-up stroke). The WAKE-UP trial[2] demonstrated the benefit of intravenous thrombolysis with alteplase in patients with stroke symptoms on waking by identifying an MRI pattern suggestive of stroke with an onset of less than 4·5 h. This method of selection increases the proportion of patients eligible for treatment, but other research[3] showed that this selection approach only identifies around 62% of patients who are within the 0–4·5 h time window. Furthermore, this approach excludes patients who might have salvageable brain tissue, despite presenting more than 4·5 h after stroke onset.

An alternative approach is to use CT perfusion or perfusion-diffusion MRI to identify patients with potentially salvageable brain tissue, regardless of time from

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*Contributed equally

†Contributed equally

‡Investigators listed in the appendix

Department of Medicine and Neurology, Melbourne Brain Centre (Prof B C V Campbell PhD, Prof M W Parsons PhD, Prof L Churilov PhD, N Yassi PhD, G Sharma MCA, A Bivard PhD, Prof B Yan DMedSci, Prof S M Davis MD, Prof G A Donnan MD) **and Department of Radiology** (Prof P M Desmond MD, Prof B Yan, Prof P J Mitchell MMed), **Royal Melbourne Hospital, and Florey Institute of Neuroscience and Mental Health** (Prof B C V Campbell, Prof H Ma PhD, Prof C F Bladin MD, N Yassi, Prof V Thijs PhD, Prof G A Donnan), **University of Melbourne, Melbourne, VIC, Australia; Department of Medicine, School of Clinical Science** (Prof H Ma) **and Department of Neurosciences, Eastern Health and Eastern Health Clinical School** (Prof H M Dewey PhD, Prof C F Bladin), **Monash University, Melbourne, VIC, Australia; Department of Neurology** (Prof P A Ringleb MD, Prof W Hacke MD) **and Department of Neuroradiology** (Prof M Bendszus MD, J A R Pfaff MD), **Ruprecht Karls University Heidelberg, Heidelberg, Germany; Department of Medicine** (Prof L Churilov) **and Department of Neurology**

(Prof V Thijs), **Austin Health, and Department of Medicine, Western Hospital, Melbourne Medical School** (Prof T Wijeratne MD), **University of Melbourne, Melbourne, VIC, Australia; Department of Neurology, Priority Research Centre for Brain and Mental Health Research, John Hunter Hospital, University of Newcastle, Newcastle, NSW, Australia** (Prof C R Levi MBBS); **Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan** (Prof C Hsu PhD); **Department of Neurology, Royal Adelaide Hospital, Adelaide, SA, Australia** (T J Kleinig PhD); **Department of Neurology, Faculty of Medicine Mannheim, University of Heidelberg, Mannheim, Germany** (Prof M Fatar MD); **Department of Neurology, Hospital CHU Lille, Lille, France** (Prof D Leys MD); **Department of Neurology, Hospital Vall d´Hebron, University of Barcelona, Barcelona, Spain** (Prof C Molina MD); **Department of Neurology, Western Health, Sunshine Hospital, Melbourne, VIC, Australia** (Prof T Wijeratne); **Department of Neurology, Helsinki University Hospital, Helsinki, Finland** (S Curtze PhD); **Department of Neurology, Auckland City Hospital, University of Auckland, Auckland, New Zealand** (Prof P A Barber PhD); **Prince of Wales Clinical School, University of New South Wales, Sydney, NSW, Australia** (Prof K S Butcher PhD); **Division of Neurology, University of Alberta, Edmonton, AB, Canada** (Prof K S Butcher); **Department of Neurology, Singapore General Hospital, National Neuroscience Institute, Singapore** (D A De Silva FRCP); **Department of Neurology, University of Erlangen-Nuremberg, Erlangen, Germany** (Prof S Schwab MD); **Department of Neurology and Neurogeriatry, Johannes Wesling Medical Centre Minden, University Hospital of Ruhr-University Bochum, Bochum, Germany** (Prof P D Schellinger MD); **Department of Neurology, Girona University Hospital, Biomedical Research Institute of Girona, Girona, Spain** (Prof J Serena PhD); **and**

Research in context

Evidence before this study

We did a systematic review of PubMed between Jan 1, 2006, and March 1, 2019, for randomised controlled trials published in English using the search terms "stroke" AND either "randomized" OR "randomised" AND either "thrombolysis" OR "alteplase" OR "tPA". In the EXTEND trial, patients randomly assigned to alteplase after automated CT or MRI perfusion imaging were found to have improvement in excellent functional outcome compared with placebo; however, the strength and precision of findings were limited by the modest sample size (n=225). The ECASS4-EXTEND trial randomly assigned 119 patients after visual assessment of perfusion-diffusion MRI, and found no statistically significant benefits of alteplase. EPITHET was a phase 2 randomised trial of alteplase 3–6 h after stroke onset using perfusion-diffusion MRI, in which 70 patients were treated within the 4·5–6 h time window. Although the overall results of the trial were neutral, in patients treated 4·5–6 h after stroke onset, thrombolysis reduced relative infarct growth and increased the rate of reperfusion. Effective reperfusion was associated with good neurological and functional outcome.

Added value of this study

This systematic review and meta-analysis of individual patient data quantifies the benefits and risks of intravenous alteplase for patients with ischaemic stroke beyond 4·5 h from when they were last known to be well. The proportion of patients with excellent functional outcome (return to all usual activities) at 3 months was significantly higher in the alteplase group than the placebo group. The proportion of patients with functional improvement (≥1 point reduction in modified Rankin Scale score) at 3 months was also significantly higher in the alteplase group than the placebo group. Although a higher proportion of patients in the alteplase group had symptomatic intracerebral haemorrhage than the placebo group, no significant difference in mortality was observed between the groups. Patients with a perfusion imaging mismatch (indicating salvageable brain tissue) detected with automated software in core laboratory analysis had significant benefit from alteplase, whereas those without perfusion mismatch detected by automated software did not, although this comparison was underpowered and formal statistical interaction was not demonstrated.

Implications of all the available evidence

Patients with ischaemic stroke 4·5–9 h after stroke onset or with wake-up stroke with evidence of salvageable brain tissue using CT perfusion or perfusion-diffusion MRI who were given intravenous alteplase have improved functional outcomes compared with those given placebo. Patients given alteplase had an increased risk of symptomatic intracerebral haemorrhage, consistent with the findings from previous trials of alteplase for stroke, but this increased risk does not offset the net benefit of thrombolysis. The benefit to risk ratio seems to be larger in patients who meet automated perfusion mismatch criteria.

stroke onset. Reperfusion beyond 4·5 h after stroke onset is associated with favourable clinical outcomes in patients with perfusion mismatch.[4–7] Patient selection on the basis of perfusion mismatch to identify treatment-responsive patients with salvageable brain tissue who have presented more than 4·5 h after stroke onset has been established for endovascular thrombectomy in patients with large vessel occlusion 6–24 h after the time they were last known to be well.[8–10] Intravenous thrombolysis could potentially treat a broader range of patients.

We did a meta-analysis of individual patient data to test the hypothesis that intravenous alteplase improves functional outcomes compared with placebo in patients with ischaemic stroke 4·5–9 h after onset or wake-up stroke who were imaged with CT perfusion or perfusion-diffusion MRI.

Methods

Search strategy and selection criteria

We did a systematic search of PubMed for randomised controlled trials published in English between Jan 1, 2006, and March 1, 2019, using the search terms "stroke" AND either "randomized" OR "randomised" AND either "thrombolysis" OR "alteplase" OR "tPA". Trials of intravenous alteplase versus placebo in adults (aged ≥18 years) with hemispheric ischaemic stroke more than 4·5 h after stroke onset or wake-up stroke who had pretreatment imaging with CT perfusion or perfusion-diffusion MRI were eligible for inclusion. Full eligibility criteria for all trials are described in the appendix. We also reviewed the reference list of a previous systematic review of thrombolysis[1] and searched ClinicalTrials.gov for interventional studies of ischaemic stroke using the keywords thrombolysis and alteplase. Patients with available data on age, pretreatment National Institutes of Health Stroke Scale (NIHSS) score, and modified Rankin Scale (mRS) score at 3 months were eligible for inclusion in the meta-analysis. Two reviewers (BCVC and NY) independently assessed articles for inclusion.

The management committees of all included trials agreed to share individual patient-level data for the purposes of this meta-analysis. Ethical approval was obtained at all participating sites for all included trials, and informed consent was obtained from all participants or their legal representative. The protocol for this study was prespecified and followed PRISMA guidelines (appendix).

Data analysis

A statistician (LC) merged the databases of the included studies on the basis of data fields prespecified in the protocol. We extracted data on treatment, age, sex, pretreatment NIHSS score, geographical location, time

post stroke onset or after wake-up stroke, atrial fibrillation, hypertension, diabetes, smoking status, NIHSS score at 72 h, mRS at 3 months, and symptomatic intracerebral haemorrhage.

For this meta-analysis, the prespecified primary outcome was the proportion of patients with excellent functional outcome (mRS score 0–1 [return to all usual activities]) at 3 months, adjusted for pretreatment clinical severity (NIHSS score) and age. Secondary outcomes were functional improvement (≥1 point reduction in mRS score [ordinal shift analysis]), with mRS categories 5 and 6 merged, at 3 months, functional independence (mRS score 0–2) at 3 months, and early neurological improvement (reduction of ≥8 points on NIHSS or reaching NIHSS score 0–1) at 72 h. All secondary outcomes were adjusted for pretreatment NIHSS score and age. Safety outcomes were symptomatic intracerebral haemorrhage defined as parenchymal haemorrhage type 2 (blood clot occupying >30% of the infarcted territory with substantial mass effect) within 36 h of treatment, combined with neurological deterioration of 4 or more NIHSS points, or death.[11] Death due to any cause at 3 months was adjusted for age and pretreatment NIHSS score.

A full description of the analyses is provided in the prespecified statistical analysis plan (appendix). Statistical analysis was done using Stata (version 15). Qualitative assessment of between trial differences including patient eligibility and assessment of bias are shown in the appendix.

Our meta-analysis followed a one-stage approach. To account for between-trial variance we used mixed-effects logistic regression models with trial and trial-by-treatment interaction terms incorporated as random effects in all models. We analysed the primary outcome and other dichotomous secondary and safety outcomes using mixed-effects logistic regression models, adjusted for baseline age and NIHSS score, to estimate adjusted odds ratios (ORs) and 95% CIs. Functional improvement was analysed using mixed-effects ordinal logistic regression (with mRS 5 and 6 categories merged), adjusted for baseline age and NIHSS score. We did preplanned subgroup analyses to assess the effects of age (≤75 *vs* >75 years and ≤80 *vs* >80 years), baseline stroke severity (NIHSS score ≤10 *vs* NIHSS score >10), time to treatment (>4·5–6·0 h, >6·0–9·0 h, or wake-up stroke), geographical region (Australia and New Zealand, Europe, or Asia), and presence of any large vessel occlusion. Large vessel occlusion was defined as occlusion of the internal carotid artery or middle cerebral artery (M1 or proximal M2), based on central imaging review, to identify patients who would be eligible for endovascular thrombectomy according to current clinical practice. Analyses were repeated in the prespecified subgroup of patients who met automated perfusion mismatch criteria. For all subgroup analyses, treatment-by-subgroup interactions were tested by including multiplicative interaction terms in respective regression models.

For analysis of the prespecified subgroup with perfusion mismatch determined by automated processing software, CT perfusion and perfusion-diffusion MRI imaging data for individual patients were uniformly reprocessed using RAPID (version 4.6; iSchemaView, Menlo Park, CA, USA) as described previously.[8,9] Although all patients in the EXTEND trial[12,13] were judged to meet automated mismatch criteria by the enrolment site, central analysis of perfusion mismatch was done to exclude imaging artefacts and to account for software evolution over the 8 years of trial recruitment. All automated output was visually verified and artefacts removed by a stroke neurologist with extensive neuroimaging analysis experience, masked to treatment allocation and all other imaging and clinical information. For CT perfusion, irreversibly injured ischaemic core was defined as a relative cerebral blood flow of less than 30% of normal brain blood flow.[14] For diffusion MRI, ischaemic core was defined as an apparent diffusion coefficient of less than 620 μm²/s.[15]

	Placebo (n=201)	Alteplase (n=213)
Age, years	72·0 (12·3)	73·2 (12·2)
Sex		
Men	116 (58%)	119 (56%)
Women	85 (42%)	94 (44%)
NIHSS score	10 (6–16)	12 (7–17)
Previously diagnosed atrial fibrillation	63 (31%)	79 (37%)
History of hypertension	134 (67%)	159 (75%)
History of diabetes	41 (20%)	49 (23%)
History of smoking	62/165 (38%)	58/179 (32%)
Geographical region		
Australia or New Zealand	110 (55%)	121 (57%)
Europe	67 (33%)	69 (32%)
Asia	24 (12%)	23 (11%)
Time from stroke onset to randomisation		
>4·5–6·0 h	49 (24%)	58 (27%)
>6·0–9·0 h	48 (24%)	50 (24%)
Wake-up stroke	104 (52%)	105 (49%)
Imaged with CT perfusion	96 (48%)	100 (47%)
Imaged with perfusion-diffusion MRI	105 (52%)	113 (53%)
Time from stroke onset* to initiation of intravenous therapy, min	413 (353–480)	417 (346–485)
Time from last known to be well to initiation of intravenous therapy, min	487 (360–655)	471 (355–649)
Large vessel occlusion	122/198 (62%)	124/205 (60%)
Ischaemic core volume† at initial imaging	8·1 (0–20·4)	8·0 (0–25·3)
Perfusion lesion volume‡ at initial imaging	64·3 (33·2–97·0)	63·9 (27·9–117·2)

Data are mean (SD), n (%), median (IQR), or n/N (%). NIHSS=National Institutes of Health Stroke Scale. *Onset time measured as the midpoint of falling asleep and waking with stroke symptoms for patients with wake-up stroke. †Relative cerebral blood flow less than 30% of normal blood flow. ‡Time to maximum >6 s.

Table 1: **Baseline characteristics of all patients**

Department of Neurology, Sapienza University of Roma, Rome, Italy (Prof D Toni MD)

Correspondence to:
Prof Geoffrey A Donnan, Department of Medicine and Neurology, Melbourne Brain Centre, Royal Melbourne Hospital, University of Melbourne, Melbourne, VIC 3050, Australia
geoffrey.donnan@unimelb.edu.au

See **Online** for appendix

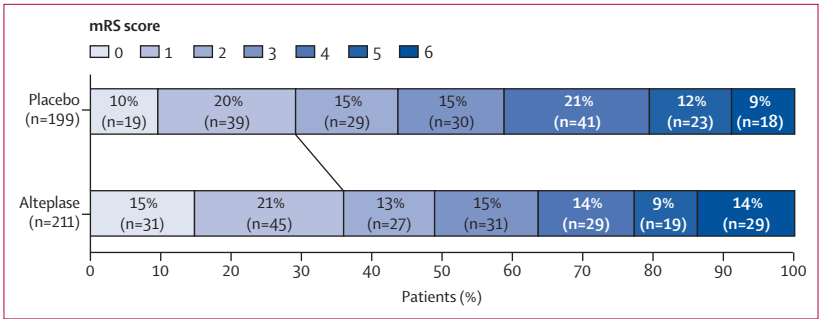

Figure 1: **mRS scores at 3 months for all patients**
mRS=modified Rankin Scale.

	Placebo (n=201)	Alteplase (n=213)	Odds ratio* (95% CI)	p value
Primary outcome				
Excellent functional outcome (mRS score 0–1) at 3 months	58/199 (29%)	76/211 (36%)	1·86 (1·15–2·99)	0·01
Secondary outcomes				
Functional improvement in mRS score at 3 months†	NA	NA	1·60 (1·12–2·27)	0·009
Functional independence (mRS score 0–2) at 3 months	87/199 (44%)	103/211 (49%)	1·74 (1·08–2·81)	0·02
Early neurological improvement at 72 h‡	31/197 (16%)	58/206 (28%)	2·54 (1·51–4·27)	<0·0001
Safety outcomes				
Death at 3 months	18/201 (9%)	29/213 (14%)	1·55 (0·81–2·97)	0·19
Symptomatic intracerebral haemorrhage§	1/201 (<1%)	10/213 (5%)	9·70 (1·23–76·55)	0·03

Data are n/N (%). mRS=modified Rankin Scale. NIHSS=National Institutes of Health Stroke Scale. NA=not applicable. *Adjusted for baseline age and NIHSS. †Reduction of ≥1 point in mRS score (with mRS categories 5 and 6 merged), analysed using ordinal logistic regression. ‡Reduction of ≥8 points on NIHSS or reaching NIHSS score 0–1 at 72 h. §Within 36h of treatment.

Table 2: **Study outcomes in all patients**

The volume of critically hypoperfused tissue (the penumbra and core combined) was estimated using CT perfusion or perfusion MRI with a time to maximum threshold of more than 6 s.[16] Time to maximum relates to the time delay in tissue enhancement after a bolus of intravenous contrast is given. Mismatch volume (ie, estimated penumbral volume) was defined as critically hypoperfused tissue volume minus ischaemic core volume. Mismatch ratio was defined as critically hypoperfused tissue volume divided by ischaemic core volume. Patients with a mismatch ratio greater than 1·2, a mismatch volume greater than 10 mL, and an ischaemic core volume less than 70 mL were considered to have perfusion mismatch. This study is registered with PROSPERO, number CRD42019128036.

Role of the funding source

There was no funding source for this study. The funders of the component EXTEND, ECASS4-EXTEND, and EPITHET studies had no role in study design, data collection, data analysis, data interpretation, or the writing of this report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Our search strategy identified three trials that met the eligibility criteria: EXTEND,[12,13] ECASS4-EXTEND,[17,18] and EPITHET.[5] The three included trials compared intravenous alteplase with placebo (0·9 mg/kg, maximum 90 mg delivered as a 10% bolus and 90% infusion over 1 h). The phase 3 EXTEND randomised controlled trial[12,13] compared alteplase with placebo in patients 4·5–9·0 h from stroke onset and patients with wake-up stroke using automated CT perfusion or MRI perfusion-diffusion mismatch selection. EXTEND recruited patients from Australasia, Taiwan, and Finland. The trial was terminated early due to loss of equipoise after the results of the WAKE-UP trial,[2] but showed that higher proportions of patients achieved excellent functional outcome with alteplase compared with placebo administered 4·5–9 h after onset or within 9 h of the midpoint of falling asleep and waking with stroke symptoms. The European ECASS4-EXTEND trial[17,18] used the same clinical eligibility criteria as EXTEND but used visual assessment of MRI perfusion-diffusion imaging. ECASS4-EXTEND was terminated early due to reduced recruitment following the positive trials[8,9] of thrombectomy in the 6–24 h treatment window. The trial demonstrated no significant benefit in the alteplase group compared with placebo. The phase 2 EPITHET trial[4] randomly assigned patients to alteplase or placebo within 3–6 h of stroke onset after perfusion-diffusion MRI, which was processed offline to determine the presence of perfusion mismatch. Only patients treated 4·5–6·0 h after stroke onset in EPITHET were included in our meta-analysis. No significant differences in the primary outcome of geometric mean infarct growth were identified between treatment groups. However, alteplase increased reperfusion, which, in turn was associated with reduced infarct growth and improved clinical outcomes.[19]

Of the 414 patients included in the three trials (mean age 73 years [SD 12·2]), 213 (51%) patients were assigned to receive alteplase and 201 (49%) to receive placebo. Baseline characteristics were largely balanced between the two groups (table 1); however, initial stroke severity was non-significantly worse in the alteplase group than the placebo group (median NIHSS score 12 [IQR 7–17] *vs* 10 [6–16]). Most patients were recruited from Australia or New Zealand, followed by Europe and Asia. Approximately half of all included patients had wake-up stroke (n=105 in the alteplase group; n=104 in the placebo group). The median time from when the patient was last known to be well to treatment in patients with wake-up stroke was 10 h 42 min (IQR 8 h 40 min–12 h 20 min). Overall, of the 403 patients with assessable angiographic imaging, 246 (61%) had large vessel occlusion that would potentially be amenable to endovascular thrombectomy.

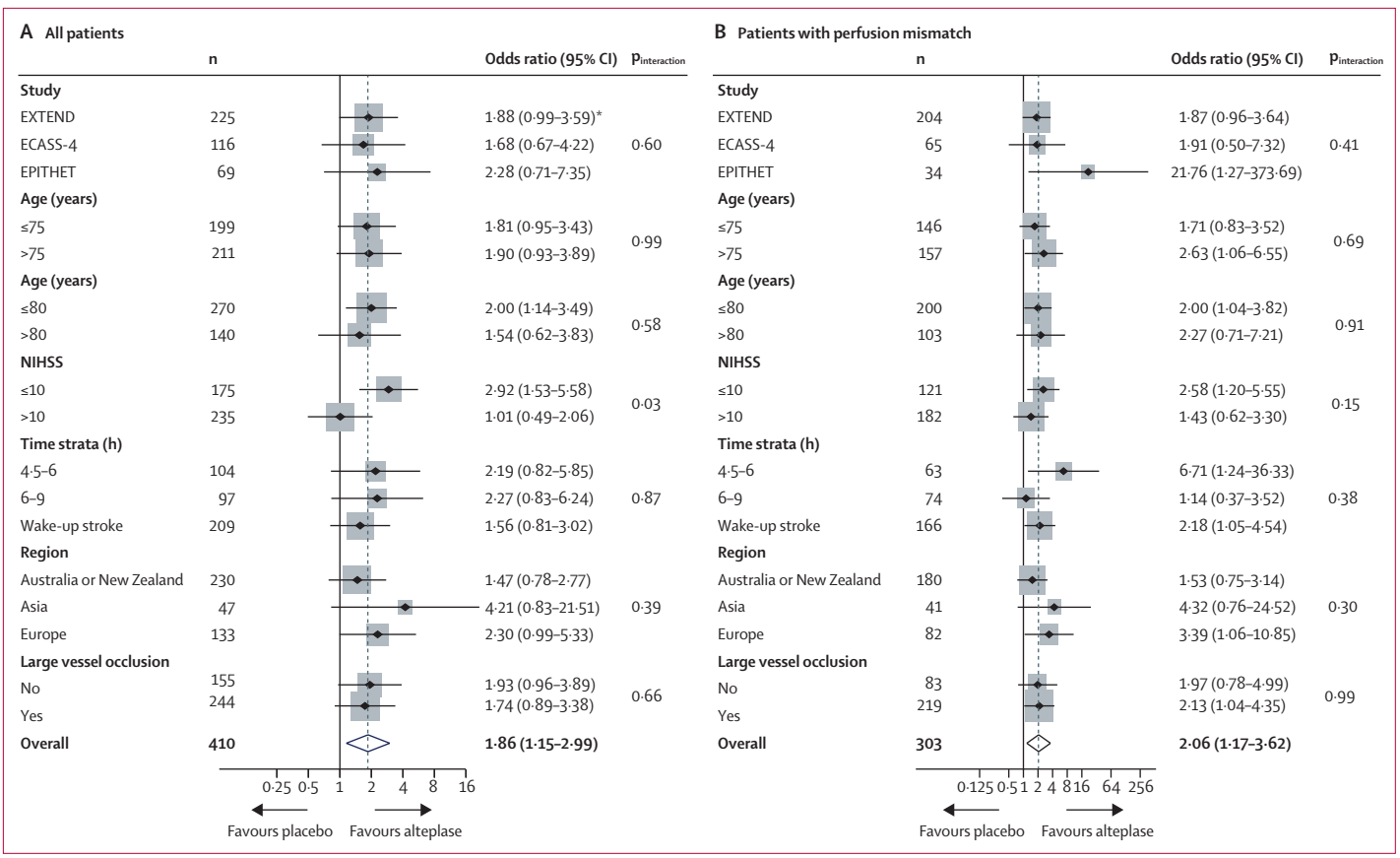

Figure 2: **Subgroup analyses**

Forest plots for the primary outcome of excellent functional outcome (modified Rankin Scale 0–1) in all patients (A) and patients with automated perfusion mismatch (B). Proportion of patients by treatment group for each subgroup are detailed in the appendix. NIHSS=National Institutes of Health Stroke Scale score. *Prespecified analysis of primary outcome for EXTEND trial used modified Poisson regression (adjusted risk ratio [for modified Rankin Scale score 0–1] 1·44, 95%CI 1·01–2·06; p=0·04).

However, 97% of patients (403 of 414) were recruited before guideline changes made in February, 2018, recommended thrombectomy in the 6–24 h window and consideration of thrombectomy was an exclusion criterion in the three trials included. The median perfusion mismatch was 47 mL (IQR 17–85) with a median volume of critical hypoperfusion of 64 mL (30–109) and relatively small core of median volume 8 mL (0–22).

Of the 414 patients included in the three trials, 211 patients in the alteplase group and 199 patients in the placebo group had mRS data available at 3 months and thus were included in the analysis of the primary outcome. 76 (36%) of 211 patients in the alteplase group achieved the primary outcome of excellent functional outcome (mRS score 0–1) at 3 months compared with 58 (29%) of 199 patients in the placebo group (adjusted odds ratio [OR] 1·86, 95% CI 1·15–2·99, p=0·011; figure 1, table 2). The proportion of patients achieving functional improvement and functional independence at 3 months, and the proportion of patients with early neurological improvement at 72 h was significantly higher in the alteplase group than the placebo group (table 2). Prespecified subgroup analyses for the primary outcome are shown in figure 2 and subgroup analyses for additional outcomes are shown in the appendix.

The number of patients with symptomatic intracerebral haemorrhage was significantly higher in the alteplase group than the placebo group (ten [5%] of 213 patients *vs* one [<1%] of 201 patients, adjusted OR 9·7, 95% CI 1·23–76·55, p=0·031). This included one patient with haemorrhagic transformation on the pretreatment CT scan (a protocol violation). However, no significant differences were identified in mortality between the alteplase and placebo groups (29 [14%] of 213 patients *vs* 18 [9%] of 201 patients, adjusted OR 1·55, 95% CI 0·81–2·96, p=0·19; table 2). All deaths are described in the appendix. Four deaths occurred in the first week after alteplase treatment due to symptomatic intracerebral haemorrhage related to treatment. One death in the alteplase group due to remote intracerebral haemorrhage that occurred on day 4 was attributed to heparin and warfarin administration. An additional death in the alteplase group occurred on day 77 due to pneumonia in a patient who had symptomatic intracerebral haemorrhage on day 1. 42 (90%) of 47 deaths were unrelated to treatment. No

	Placebo (n=152)	Alteplase (n=152)
Age, years	72·1 (12·3)	73·2 (13·1)
Sex		
Men	91 (60%)	86 (57%)
Women	61 (40%)	66 (43%)
NIHSS score	11 (7–17)	12 (7–17)
Previously diagnosed atrial fibrillation	53 (35%)	60 (40%)
History of hypertension	95 (63%)	113 (74%)
History of diabetes	28 (19%)	37 (24%)
History of smoking	52/119 (44%)	40/121 (33%)
Geographical region		
Australia or New Zealand	91 (60%)	89 (59%)
Europe	40 (26%)	43 (28%)
Asia	21 (14%)	20 (13%)
Stroke onset to randomisation time		
>4·5–6·0 h	31 (20%)	33 (22%)
>6·0–9·0 h	37 (24%)	37 (24%)
Wake-up stroke	84 (55%)	82 (54%)
Imaged with CT perfusion	90 (59%)	89 (59%)
Imaged with perfusion-diffusion MRI	62 (41%)	63 (41%)
Time from stroke onset* to initiation of intravenous therapy, min	422 (355–492)	425 (351–490)
Time from last known to be well to initiation of intravenous therapy, min	503 (374–657)	489 (374–670)
Large vessel occlusion	113/152 (74%)	107/151 (71%)
Ischaemic core volume† at initial imaging	8·9 (0–20·0)	6·2 (0–22·3)
Perfusion lesion volume‡ at initial imaging	75·1 (47·0–104·9)	74·0 (40·2–117·2)

Data are mean (SD), n (%), median (IQR), or n/N (%). NIHSS=National Institutes of Health Stroke Scale. *Onset time measured as the midpoint of falling asleep and waking with stroke symptoms for patients with wake-up stroke. †Relative cerebral blood flow less than 30% of normal blood flow. ‡Time to maximum >6 s.

Table 3: **Baseline characteristics of patients with automated perfusion mismatch**

significant differences were identified in the proportion of patients with combined mRS scores of 5 or 6 (death and requirement for constant nursing care): 48 (23%) of 211 patients in the alteplase group versus 41 (21%) of 199 patients in the placebo group (adjusted OR 1·03, 95% CI 0·62–1·74, p=0·897).

Central adjudication of perfusion mismatch status using automated RAPID software was possible in 405 patients (raw imaging data were not available for six patients in the alteplase group and three patients in the placebo group). After central adjudication of perfusion mismatch status in these 405 patients, 304 patients had perfusion mismatch (152 [73%] of 207 patients in the alteplase group and 152 [77%] of 198 patients in the placebo group). Baseline characteristics of patients with automated perfusion mismatch were balanced between the groups (table 3). Among the 303 patients with automated perfusion mismatch who had available mRS scores at 3 months, the proportion of patients who achieved excellent functional outcome was higher in the alteplase group than the placebo group (55 [36%] of 152 patients *vs* 39 [26%] of 151 patients; adjusted OR 2·06, 95% CI 1·17–3·62, p=0·012; figure 3, table 4), and the point estimate of the effect size was larger in this subgroup analysis than the intention-to-treat analysis. Among patients without mismatch, no significant differences were observed in the proportion of patients who achieved excellent functional outcome between the alteplase and placebo groups (adjusted OR 1·22, 95% CI 0·48–3·10, p=0·68; treatment by mismatch status interaction p=0·43). Similarly, among patients with mismatch, significant improvements in all secondary outcomes were observed in the alteplase group (table 4), whereas no significant differences were identified between the treatment groups among patients without mismatch (appendix). Safety outcomes in patients with perfusion mismatch were similar to those for all patients. Among patients with perfusion mismatch, the proportion of patients with an mRS score of 5 or 6 in the alteplase and placebo groups was similar (34 [23%] of 152 patients *vs* 36 [23%] of 151 patients; adjusted OR 0·83, 95% CI 0·46–1·53, p=0·557).

Discussion

This meta-analysis of pooled individual patient-level data has shown that thrombolysis with intravenous alteplase in patients with acute ischaemic stroke 4·5–9 h after stroke onset or wake-up stroke, who were imaged with CT perfusion or perfusion-diffusion MRI, improves functional outcomes compared with placebo. The frequency of symptomatic intracerebral haemorrhage was around 4% higher in the alteplase group than the placebo group, which is consistent with the results of previous trials[1] of alteplase 0–4·5 h after stroke. However, this did not negate the benefit of alteplase in ordinal analysis of the mRS, which accounts for transitions across the disability spectrum. Mortality was not significantly increased with alteplase and the proportion of patients who had an mRS score of 5 or 6 (ie, dead or requiring nursing home care) did not differ significantly between groups. Although no statistically significant interaction was identified between mismatch status and treatment effect, the benefit of alteplase in terms of functional outcome and independence, and neurological improvement was significant in patients who met automated perfusion mismatch criteria, but did not reach statistical significance in patients without perfusion mismatch.

The benefits of alteplase observed in this analysis compare favourably with those reported within 4·5 h of stroke onset. The adjusted OR for achieving an mRS score 0–1 among all patients was 1·86 (95% CI 1·15–2·99) and 2·06 (1·17–3·62) for patients with automated perfusion mismatch, compared with an adjusted OR of 1·75

(1·35–2·27) for alteplase administered 0–3 h from stroke onset and 1·26 (1·05–1·51) for alteplase administered 3–4·5 h from stroke onset, as reported previously.[1] The absolute increase in the proportion of patients who achieved excellent functional outcome in this pooled analysis was 7% higher in the alteplase group than the placebo group (10% in patients with perfusion mismatch). However, these unadjusted proportions are likely to underestimate the true benefit since there was a trend towards older age and greater clinical severity in the patients given alteplase. By comparison, alteplase administered 0–3 h after stroke onset improved excellent functional outcome by 9·8%. This reduced to 5·2% when administered at 3–4·5 h.[1] In the WAKE-UP trial,[2] which administered alteplase based on MRI evidence of onset at less than 4·5 h, the unadjusted improvement in excellent outcome was 11·5% and the adjusted OR was 1·61 (95% CI 1·09–2·36). The treatment effect with alteplase was numerically lower than that observed with endovascular thrombectomy (18% difference in mRS score 0–1).[8,9] This difference might reflect the more effective reperfusion achieved with endovascular thrombectomy. The mismatch volume in this analysis was also approximately half that reported in previous thrombectomy trials,[8,9] which might have reduced the potential benefit of reperfusion. For patients with wake-up stroke, both EXTEND and ECASS4-EXTEND diverged from the standard definition of stroke onset (ie, time the patient was last known to be well). The treatment window for patients with wake-up stroke in EXTEND and ECASS4-EXTEND was 9 h after the midpoint of the time they fell asleep to the time they woke with symptoms. Using the standard definition of onset this led to patients being treated up to almost 16 h after the time the patient was last known to be well.

The risks of alteplase in the extended time window were similar to those for the standard 0–4·5 h treatment window. Symptomatic intracranial haemorrhage occurred in 4·7% of patients compared with 3·4% in a meta-analysis of 0–4·5 h alteplase trials[20] using the same definition. Fatal intracerebral haemorrhage occurred in 2·3% of patients in this analysis compared with 2·6% in the 0–4·5 h data.[1] Automated perfusion mismatch selection did not seem to alter the risk of symptomatic intracerebral haemorrhage in the alteplase group compared with all patients. However, detailed analysis of the cause and timing of death indicated that 42 (90%) of 47 deaths were not related to alteplase (appendix). No significant difference in the proportion of patients with an mRS score of 5 or 6 (death and requirement for constant nursing care) was identified between treatment groups. The absence of mortality benefit with thrombolysis in this analysis is consistent with trials of alteplase 0–4·5 h after stroke onset.[1]

The three trials[5,13,18] included in this meta-analysis demonstrated consistent treatment effects with minimal heterogeneity. This pooled analysis substantially strengthens the results of the EXTEND trial, which demonstrated statistically significant improvement in excellent functional outcome (mRS score 0–1) and functional independence (mRS score 0–2) but did not reach significance for the ordinal functional improvement outcome. The magnitude of benefit using the ordinal outcome indicates that the benefit of increased excellent outcome is not offset by an increase in poor outcomes,

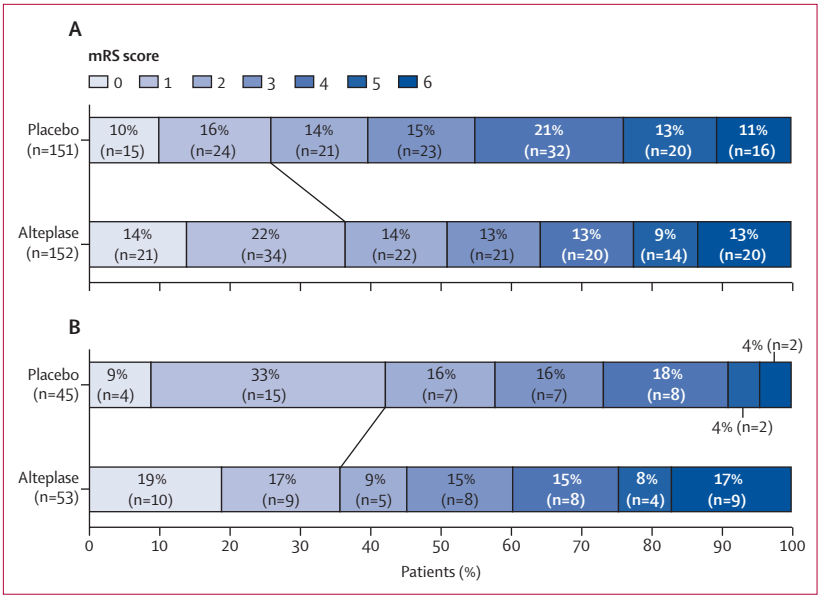

Figure 3: **mRS score at 3 months by perfusion mismatch subgroup**
mRS score for patients with automated perfusion mismatch (A), and patients without automated perfusion mismatch (B). Among patients with mismatch, one patient in the placebo group was excluded because they did not have mRS assessment at 3 months. Among patients without mismatch, one patient in the placebo group and two patients in the alteplase group were excluded because they did not have mRS assessment at 3 months. Imaging data were not available for six patients in the alteplase group and three patients in the placebo group; thus perfusion mismatch status could not be determined. mRS=modified Rankin Scale.

	Placebo (n=152)	Alteplase (n=152)	Odds ratio (95% CI)*	p value
Primary outcome				
Excellent outcome (mRS score 0–1) at 3 months	39/151 (26%)	55/152 (36%)	2·06 (1·17–3·62)	0·012
Secondary outcomes				
Functional improvement in mRS score at 3 months†	NA	NA	1·68 (1·11–2·53)	0·014
Functional independence (mRS score 0–2) at 3 months	60/151 (40%)	77/152 (51%)	2·22 (1·25–3·94)	0·006
Early neurological improvement at 72 h‡	26/152 (17%)	44/148 (30%)	2·26 (1·26–4·03)	0·006
Safety outcomes				
Death at 3 months	16/152 (11%)	20/152 (13%)	1·28 (0·60–2·73)	0·52
Symptomatic intracerebral haemorrhage§	1/152 (1%)	7/152 (5%)	7·29 (0·88–60·18)	0·07

Data are n/N (%). mRS=modified Rankin Scale. NIHSS=National Institutes of Health Stroke Scale. NA=not applicable. One patient in the placebo group did not have available mRS data at 3 months and thus was excluded from the analysis of the primary outcome and selected secondary outcomes. *Adjusted for baseline age and NIHSS. †Reduction of ≥1 point in mRS score (with mRS categories 5 and 6 merged), analysed using ordinal logistic regression. ‡Reduction of ≥8 points on NIHSS or reaching NIHSS score 0–1 at 72 h. §Within 36 h of treatment.

Table 4: **Study outcomes in patients with automated perfusion mismatch (n=304)**

despite the increase in symptomatic intracerebral haemorrhage.

Automated imaging selection was successfully used in previous trials[8,9] of extended time windows for endovascular thrombectomy and in EXTEND.[12] This provides objective thresholds to standardise imaging assessment. Applying automated processing in this analysis excluded around 24% of patients, mostly with small perfusion lesions with less than 10 mL mismatch. The analysis was underpowered to detect a statistical interaction between mismatch status and treatment effect. However, the magnitude of difference in point estimates suggests that the effect is driven by patients meeting perfusion mismatch criteria.

Limitations of this study include the early termination of two of the three included trials (EXTEND and ECASS4-EXTEND), which limited the overall sample size. In both trials, however, termination was due to external factors, which reduces the potential bias. The requirement for perfusion imaging might impede implementation in some regions. However, the CT perfusion imaging selection used in the majority of patients included in the EXTEND trial is more generally available than urgent MRI. Automated software is commercially available but can be costly in resource-restricted environments. Our results suggest that automated assessment of mismatch is more reliable for the identification of treatment responders than visual assessment, consistent with previous studies.[21,22] Physician oversight is, however, still required for the interpretation of automated output. An MRI-based selection approach would allow either the diffusion-FLAIR mismatch approach, as used in the WAKE-UP trial, or perfusion-diffusion mismatch selection, which would maximise patient eligibility for alteplase treatment. Perfusion mismatch selection allows treatment of patients beyond 4·5 h who would be excluded by the diffusion-FLAIR approach. However, patients with lacunar stroke would not meet perfusion mismatch criteria, but were found to benefit from thrombolysis in WAKE-UP.[23] CT perfusion can identify around 50% of patients with lacunar stroke,[24] but no data are available on thrombolysis of lacunar stroke more than 4·5 h from stroke onset based on CT perfusion. The trials included in this meta-analysis were largely done before the publication of evidence supporting endovascular thrombectomy in patients with large vessel occlusion 6–24 h after stroke onset. Because recruitment largely preceded the evidence for thrombectomy, only one patient in this analysis had thrombectomy (a protocol violation). However, in current practice, 61% of patients in this analysis would now be eligible for thrombectomy on the basis of the presence of a proximal large vessel occlusion. The combined use of thrombolysis and endovascular thrombectomy in an extended time window is currently being investigated in an ongoing trial (NCT03785678). However, many institutions would be capable of delivering thrombolysis but not endovascular thrombectomy. In the absence of immediate access to endovascular thrombectomy, this meta-analysis supports the use of intravenous thrombolysis in patients with perfusion mismatch 4·5–9 h from stroke onset or with symptoms on waking, regardless of the presence of large vessel occlusion. For patients without large vessel occlusion, our data expand the proportion of patients with ischaemic stroke who can receive reperfusion therapies.

In conclusion, alteplase improved functional outcomes in patients with acute ischaemic stroke in an extended time window when perfusion imaging was favourable. The benefit was evident across a range of patient subgroups and geographical locations. The risk of symptomatic intracerebral haemorrhage was consistent with that observed in previous trials of stroke thrombolysis with alteplase in the 0–4·5 h treatment window and did not offset the overall benefits in functional outcome. This pooled analysis provides strong evidence in support of thrombolysis for patients with favourable perfusion imaging 4·5–9 h after stroke, including patients with wake-up stroke.

Contributors

BCVC, HM, PAR, LC, WH, SMD, and GAD developed the study protocol and drafted the manuscript. Imaging analysis was done by BCVC, GS, AB, and NY. All authors collected data and edited the manuscript.

Declaration of interests

PAR reports grants from Boehringer Ingelheim during the conduct of the study, and personal fees from Boehringer Ingelheim, Bayer, and Pfizer, outside the submitted work. MWP reports reimbursement for travel expenses from Boehringer Ingelheim, and collaborated with Apollo Medical Imaging for research purposes, outside the submitted work. MB reports personal fees from Boehringer Ingelheim, Merck, Bayer, Teva, BBraun, Springer, Vascular Dynamics, and Grifols; grants and personal fees from Novartis, Codman, and Guerbet; and grants from Siemens, Hopp Foundation, Deutsche Forschungsgemeinschaft, the European Union, Stryker, and Medtronic, outside the submitted work. DL reports consultancy fees paid to his institution from Bristol-Myers Squibb and Pfizer, Boehringer Ingelheim, and Bayer, outside the submitted work, and is vice-editor of the European Stroke Journal. HMD reports reports reimbursement for travel expenses and accommodation from Boehringer Ingelheim. CFB reports personal fees, reimbursement for travel expenses and accommodation, and unrestricted educational funding from Boehringer Ingelheim, outside the submitted work. JARP reports personal fees from Stryker and MicroVention, outside the submitted work. PDS reports personal fees from Boehringer Ingelheim, outside the submitted work. DT reports personal fees from Boehringer Ingelheim, Bayer, Pfizer, Daiichi Sankyo, and Abbott, outside the submitted work. VT reports personal fees and reimbursement for conference registration fees, travel expenses and accommodation from Boehringer Ingelheim, Bayer, Pfizer, and BMS; and personal fees from Amgen, Medtronic, and Takeda, outside the submitted work. WH reports grants from Boehringer Ingelheim. SMD reports personal fees from Bayer, Boehringer Ingelheim, Medtronic, and Tide Pharmaceuticals, outside the submitted work. GAD reports grants from the Australian National Health and Medical Research Council; and personal fees from Allergan, Amgen, Bayer, Boehringer Ingelheim, Pfizer and Servier, outside the submitted work. All other authors declare no competing interests.

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