JAMA Network Open

Original Investigation | Neurology

Efficacy and Safety of Recombinant Human Prourokinase in the Treatment of Acute Ischemic Stroke Within 4.5 Hours of Stroke Onset

A Phase 3 Randomized Clinical Trial

Haiqing Song, MD, PhD; Yuan Wang, MD; Qingfeng Ma, MD, PhD; Wuwei Feng, MD, PhD; Rui Liu, MMed; Xiaomei Lv, MSc; Lijuan Huang, BSc; Yifan Li, MSc; Yi Yang, MD, PhD; Deqin Geng, MMed; Jianguo Zhu, MD; Yan Wei, MMed; Huisheng Chen, MD, PhD; Runxiu Zhu, MD; Qijin Zhai, MD; Jing Guo, MBBS; Bo Liu, MD; Shigang Zhao, MD, PhD; Yuping Wang, MD, PhD; for the PROST collaborative group

Abstract

IMPORTANCE Recombinant human prourokinase (rhPro-UK) is a thrombolytic agent that has shown promising findings in a phase 2 clinical trial in patients with acute ischemic stroke (AIS).

OBJECTIVE To evaluate the efficacy and safety of rhPro-UK thrombolysis within 4.5 hours of symptom onset in patients with AIS.

DESIGN, SETTING, AND PARTICIPANTS This randomized, alteplase-controlled, open-label, phase 3 clinical trial was conducted from May 2018 to May 2020 at 35 medical centers in China. A total of 684 patients were screened and 674 patients were enrolled. Included patients were aged 18 to 80 years with a diagnosis of AIS and received treatment within 4.5 hours of stroke onset. Data were analyzed from June to October 2020.

INTERVENTIONS Eligible patients were randomly assigned (1:1) to receive intravenous rhPro-UK or alteplase.

MAIN OUTCOMES AND MEASURES The primary objective was to assess whether rhPro-UK was noninferior to alteplase. The noninferiority margin was a between-group difference of less than 10%. The primary outcome was a modified Rankin Scale score of 0 to 1 at 90 days.

RESULTS Among 663 patients in the modified intention-to-treat population (mean [SD] age, 61.00 [10.20] years; 161 females [24.3%]), there were 330 patients in the rhPro-UK group and 333 patients in the alteplase group. The median (IQR) baseline National Institutes of Health Stroke Scale score was 6.00 (5.00-9.00). There were 23 deaths, and 619 patients (93.4%) completed the 3-month follow-up. The primary outcome occurred in 215 patients (65.2%) in the rhPro-UK group and 214 patients (64.3%) in the alteplase group (risk difference, 0.89; 95.4% CI, −6.52 to 8.29). Symptomatic intracerebral hemorrhage occurred in 5 patients (1.5%) in the rhPro-UK group and 6 patients (1.8%) in the alteplase group ($P > .99$). Systemic bleeding within 90 days occurred more frequently in the alteplase group (141 patients [42.2%]) than the rhPro-UK group (85 patients [25.8%]) ($P < .001$). By 90 days, 5 thrombolysis-related deaths each had occurred in the rhPro-UK group (1.5%) and alteplase group (1.5%) ($P > .99$).

CONCLUSIONS AND RELEVANCE This study found that intravenous rhPro-UK within 4.5 hours of AIS onset was noninferior to alteplase. The rhPro-UK group showed a similar rate of symptomatic ICH but fewer cases of systemic bleeding than the alteplase group.

(continued)

Key Points

Question Is intravenous recombinant human prourokinase (rhPro-UK) thrombolysis within 4.5 hours after the onset of acute ischemic stroke (AIS) safe and effective for patients?

Findings In this phase 3 randomized clinical trial of 663 patients with AIS, 65.2% and 64.3% of patients in rhPro-UK and alteplase groups, respectively, achieved favorable outcomes (modified Rankin scale score, 0 or 1) at 90 days. Moreover, rhPro-UK significantly reduced the risk of systemic bleeding.

Meaning This study found that intravenous thrombolysis with rhPro-UK within 4.5 hours of AIS onset showed similar efficacy and safety as alteplase treatment.

+ Visual Abstract

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

Open Access. This is an open access article distributed under the terms of the CC-BY-NC-ND License.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

Abstract (continued)

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT03541668

JAMA Network Open. 2023;6(7):e2325415. doi:10.1001/jamanetworkopen.2023.25415

Introduction

Ischemic stroke accounts for 69.6% to 70.8% of all strokes. Intravenous thrombolysis (IVT) within 4.5 hours of symptom onset is the first-line therapy in patients with acute ischemic stroke (AIS), and recombinant tissue plasminogen activator (alteplase) is the preferred thrombolytic agent.[1]

As a specific plasminogen activator that mainly acts on fibrin at the thrombus site, prourokinase does not form covalent complexes with protease inhibitors in plasma.[2] Therefore, prourokinase therapies have a potential advantage of less systemic bleeding and intracranial hemorrhage (ICH).[3-5] In animal stroke models, intravenous prourokinase leads to improved recanalization, decreased infarct volume, and reduced neurological deficit, with a reduced risk of systemic bleeding and ICH.[6,7]

With an amino acid sequence identical to that of natural prourokinase, recombinant human prourokinase (rhPro-UK) is efficacious in treating acute myocardial infarction (AMI).[8-10] In 2011, the National Medical Products Administration of China approved intravenous rhPro-UK for the treatment of AMI. Its use can be fully guaranteed, not only at large hospitals in big cities, but also at primary medical institutions. For patients weighing more than 56 kg, the price of single-treatment rhPro-UK is $787, which is half the price of r-tPA ($1577). Recently, rhPro-UK has been used for the management of AMI in 2900 hospitals in more than 300 cities in China. Increased ease of administration (uniform dosage and shorter maintenance infusion) and cost-effectiveness are advantages of rhPro-UK.

We conducted a randomized phase 2 clinical trial in 2016 using an open-label, positive-control, and multicenter design (Chinese Clinical Trial Registry Identifier: ChiCTR1800016519). Results suggested that rhPro-UK IVT within 4.5 hours after stroke onset was effective.[11] Findings also showed that treatment with 35 mg of rhPro-UK was as effective as that with 50 mg of rhPro-UK but had slightly better safety, prompting us to initiate a multicenter phase 3 study. The Efficacy and Safety of Recombinant Human Prourokinase in the Treatment of Acute Ischemic Stroke Within 4.5 Hours of Stroke Onset (PROST) trial was designed to be a noninferiority trial comparing the effectiveness of rhPro-UK with that of alteplase administered within 4.5 hours of AIS onset.

Methods

Study Design and Participants

The PROST trial is a randomized, alteplase-controlled, open-label, phase 3 clinical study to evaluate the efficacy and safety of rhPro-UK vs alteplase thrombolysis within 4.5 hours of symptom onset in patients with AIS. The trial protocol is available in Supplement 1. Patients were eligible if they were aged 18 to 80 years, had been diagnosed with AIS, had experienced stroke onset within 4.5 hours, and had a stroke severity score of 4 to 25 on the National Institutes of Health Stroke Scale (NIHSS). Cerebral computed tomography (CT) was required before randomization to exclude patients with ICH or massive ischemic infarction (defined as hypodensity >1/3 of middle cerebral artery territory on CT). Patients with stroke or myocardial infarction within the previous 3 months, a history of ICH, or planned endovascular treatment were also excluded. The exclusion criteria were mainly based on current contraindications[12] and were determined a priori.

The trial was approved by the institutional review board at each participating site. Written informed consent was obtained from patients or their legal representatives before enrolment. This trial was conducted in accordance with the Declaration of Helsinki. The study was registered with

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

ClinicalTrials.gov (NCT03541668) and followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

Randomization and Masking

Eligible patients were randomly assigned at a ratio of 1:1 to receive rhPro-UK (35 mg, administered intravenously with a bolus of 15 mg within 3 minutes and the remainder by continuous infusion within 30 minutes) or alteplase (0.9 mg/kg [maximum 90 mg], with 10% administered intravenously as a bolus, followed by 90% infusion within 1 hour). An interactive network web system using computer-generated random numbers created by an independent biostatistician was used at all participating centers.

This was an open-label trial, but patients at each center were assessed by independent investigators who were blinded to the group assignment. End point assessments were done on day 90, with a separate case report of the modified Rankin Scale (mRS), NIHSS, and Barthel Index (which measures 10 basic activities of daily living or mobility items; scale range, 0-100) scores provided to investigators to ensure that they were blinded to the allocation of patients. Scans from CT, magnetic resonance imaging (MRI), or both were analyzed independently by radiologists at each center, who had no access to patient case report forms and were masked to treatment allocation.

Study Procedures

Alteplase or rhPro-UK was the sole thrombolysis treatment in each study group and was not combined with other thrombolytic drugs. Antiplatelet therapy was administered when necessary but not within 24 hours of the start of thrombolysis. Except for patients showing pulmonary embolism, atrial fibrillation, or deep venous thrombosis of the lower extremity, anticoagulants were not used.

Neurological deficit was measured with the NIHSS. Functional outcome was measured with the mRS. Patient ability to perform daily activities was measured with the Barthel Index. These scales were assessed by certified or trained investigators at baseline and at 24 hours, 7 days, 30 days, and 90 days after treatment. The NIHSS score was also assessed 2 hours after treatment. Telephone visits were used to assess the mRS score when the patient was discharged and unable to return to the hospital for an in-person visit. Vital signs were closely monitored for the first 24 hours. A brain CT was performed at baseline and 24 hours after randomization. An additional CT or MRI was performed at the discretion of local physicians.

An interim analysis was performed after the 340th enrolled patient had completed the visit on day 90. An independent data monitoring committee (IDMC) regularly monitored the safety of the trial and made recommendations based on efficacy and safety results of the interim analysis. IDMC members did not participate in the design or conduct of the trial.

Outcome Assessments

The primary outcome was the proportion of patients with an excellent functional outcome (defined as an mRS score of 0-1) at 90 days. Secondary outcomes included major neurological improvement (defined as a reduction in NIHSS score of ≥4 or a score of 0-1) at 24 hours, functional independence (defined as an mRS score of 0-2) at 90 days, and a Barthel Index score of 75 to 100 at 90 days after treatment. Safety outcomes were all-cause death within 7 days and 90 days, systemic bleeding and ICH within 90 days, symptomatic ICH (defined by the Safe Implementation of Thrombolysis in Stroke-Monitoring Study [SITS-MOST],[13] European Cooperative Acute Stroke Study [ECASS] III,[14] and National Institute of Neurological Disorders and Stroke [NINDS][15]), and recurrent ischemic stroke within 7 days.

Statistical Analysis

The noninferiority margin in our study was defined as 10%, and the statistical power was 80%. The noninferiority margin of a 10% absolute difference was selected for the trial because this was viewed informally as a clinically significant difference by the main investigators and approved by clinical and

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

statistical experts. A noninferiority test with a 1-sided significance level of .025 was used. Considering a 20% loss to follow-up rate, 340 participants were required for each group, with a total of 680 participants for the trial.

An interim analysis was conducted after completion of follow-up on day 90 of the 340th enrolled patient. The sponsor considered IDMC recommendations at the interim analysis meeting and decided to continue the clinical trial without adjusting the sample size. According to the Lan-DeMets consumption function combined with the O'Brien-Fleming efficacy boundary, the type I error rate in the interim analysis was controlled at 0.002 on 1 side. To ensure that the overall type I error rate of the whole test was controlled at 0.025 on 1 side and the type I error rate of the final analysis was controlled at 0.023 on 1 side, the noninferiority criterion for the final analysis was that the main difference in primary outcome between groups on 1 side of the 95.4% CI was greater than −10%.

The main analyses of efficacy outcomes were the modified intention-to-treat (mITT) and per-protocol analyses. Missing outcome data were replaced with the last observation carried forward. The per-protocol analysis excluded patients who were disqualified according to protocol quality control, such as patients who did not use the study drug strictly in accordance with the protocol and patients whose records did not contain primary outcome data.

As a post hoc analysis, multiple imputation and available case analysis for the primary outcome were conducted as sensitivity analyses. Subgroup analyses were prespecified for the primary outcome according to age (≤60 or >60 years), sex (male or female), baseline NIHSS score (4-7, 8-14, or ≥15), and treatment window (≤3 hours or >3 to 4.5 hours).

Descriptive statistics were used for demographic and baseline characteristics. To assess outcomes, the χ^2 test was used, and the 95.4% CI of the difference between groups in the proportion of patients with a 90-day mRS score of 0 to 1 was calculated. The exact probability method was used to assess safety outcomes. Study data were analyzed by an independent biostatistician using SAS statistical software version 9.4 (SAS Institute Inc) (Supplement 1). Data were analyzed from June to October 2020.

Results

From May 2018 through May 2020, a total of 684 patients were screened across 35 medical centers in China. We deemed 10 patients ineligible based on criteria. The remaining 674 patients were randomly allocated to the rhPro-UK group (337 patients [50.0%]) or alteplase group (337 patients) (**Figure 1**). Among these patients, 10 individuals were excluded from the mITT analysis because they did not undergo thrombolysis with rhPro-UK or alteplase (7 patients in the rhPro-UK group and 3 patients in the alteplase group). A patient in the alteplase group diagnosed with mitochondrial encephalopathy, a stroke mimic, was also excluded from the mITT analysis. Therefore, 663 patients constituted the mITT population (mean [SD] age, 61.00 [10.20] years; 161 females [24.3%]), including 330 patients in the rhPro-UK group and 333 patients in the alteplase group. There were 23 deaths, and 619 patients (93.4%) completed the 3-month follow-up (eTable 1 in Supplement 2).

Patient baseline characteristics are shown in **Table 1**. The mean (SD) age of patients was 61.44 (10.20) years in the rhPro-UK group and 60.57 (10.20) years in the alteplase group. The median (IQR) interval from onset to thrombolysis was 3.43 (2.70-4.03) hours in the rhPro-UK group and 3.50 (2.75-4.13) hours in the alteplase group. The median (IQR) NIHSS score was 6.00 (5.00-9.00) overall, 6.00 (5.00-10.00) in the rhPro-UK group, and 6.00 (5.00-9.00) in the alteplase group. The occurrence of stroke risk factors was similar between groups.

Efficacy

In the mITT analysis, 215 patients (65.2%) in the rhPro-UK group and 214 patients (64.3%) in the alteplase group achieved the primary outcome of an mRS score of 0 to 1 at 90 days (risk difference [RD], 0.89; 95.4% CI, −6.52 to 8.29; P = .81) (**Table 2** and **Figure 2**; eTable 2 in Supplement 2). The

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

primary outcome using multiple imputation did not differ between groups (RD, 0.34; 95.4% CI, −5.13 to 5.81; *P* = .90) (eTable 3 in Supplement 2). Sensitivity analysis based on only observed data showed a similar result (RD, 1.14; 95.4% CI, −6.31 to 8.59; *P* = .76) (eTable 4 in Supplement 2). Prespecified subgroup analyses are shown in the eFigure in Supplement 2.

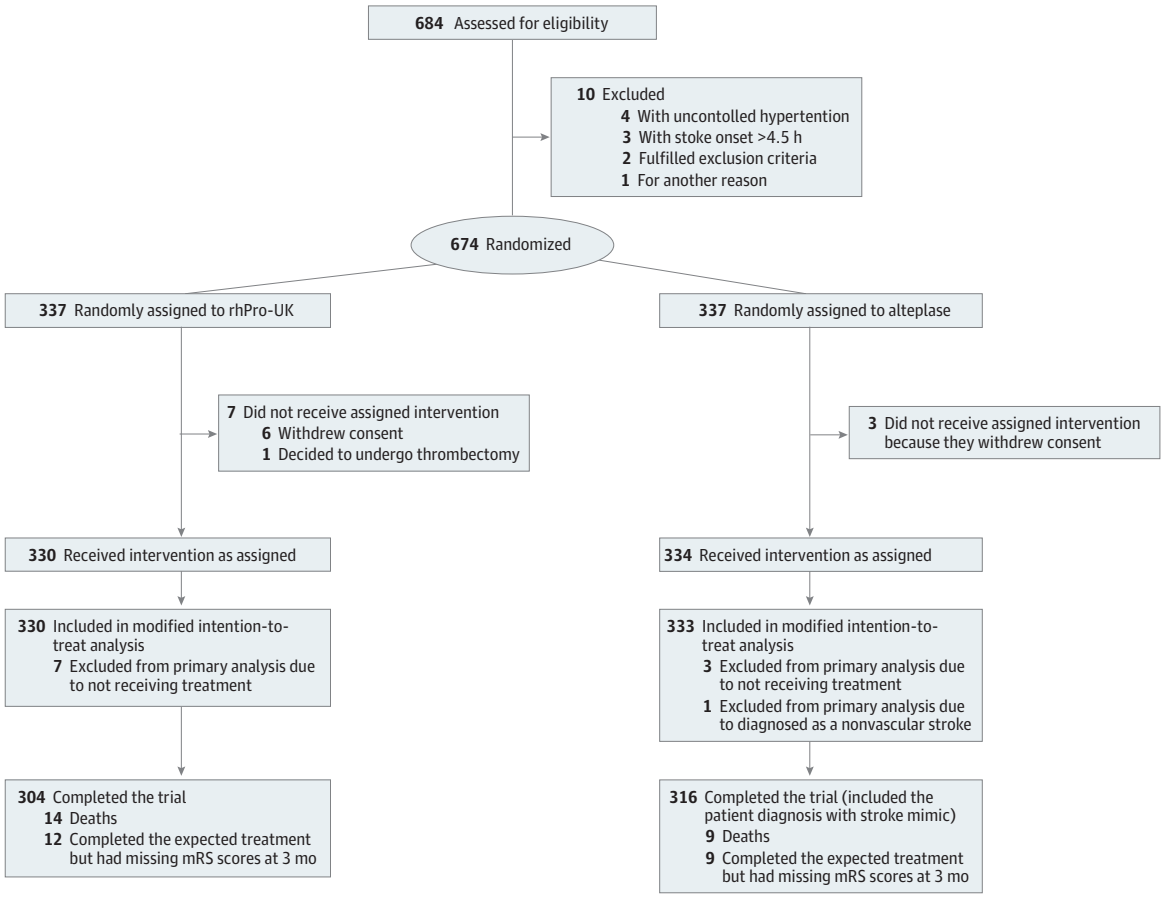
Major neurological improvement at 24 hours was observed in 132 patients (40.0%) in the rhPro-UK group and 142 patients (42.6%) in the alteplase group (RD, −2.64; 95% CI, −10.14 to 4.85; *P* = .49). By 90 days, 251 patients (76.1%) in the rhPro-UK group and 250 patients (75.1%) in the alteplase group had achieved an mRS score of 0 to 2 (RD, 0.99; 95% CI, −5.56 to 7.53; *P* = .77). A Barthel Index score of 75 to 100 was obtained in 272 patients (82.4%) in the rhPro-UK group and 271 patients (81.4%) in the alteplase group (RD, 1.04; 95% CI, −4.82 to 6.90; *P* = .73).

The per-protocol analysis of the primary outcome included 639 patients (318 patients in the rhPro-UK group and 321 patients in the alteplase group). An mRS score of 0 to 1 at 90 days was observed in 210 patients (66.0%) in the rhPro-UK group and 212 patients (66.0%) in the alteplase group (RD, −0.01; 95.4% CI, −7.48 to 7.47; *P* = .99). Groups showed no significant difference in secondary outcomes, consistent with results of the mITT analysis (Table 2).

Safety

The patient diagnosed with stroke mimic, who was excluded from the mITT analysis, could be analyzed in the safety data set, which included 330 patients in the rhPro-UK group and 334 patients in the alteplase group. Death within 90 days was reported in 14 patients (4.2%) in the rhPro-UK

Figure 1. Trial Flowchart



mRS indicates modified Rankin Scale; rhPro-UK, recombinant human prourokinase.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

group and in 9 patients (2.7%) in the alteplase group (P = .30). The rhPro-UK group had 5 deaths attributed to thrombolysis (1.5%), as did the alteplase group (1.5%) (P > .99); 13 remaining deaths were considered unrelated to thrombolysis. In the rhPro-UK group, 5 deaths were attributed to the study drug, of which 4 deaths were caused by fatal symptomatic ICH and 1 death had an unknown cause, as was the case in the alteplase group.

Table 1. Baseline Patient Characteristics

Characteristic	Patients, No. (%) (N = 663)	
	rhPro-UK group (n = 330)	Alteplase group (n = 333)
Sex		
Male	256 (77.6)	246 (73.9)
Female	74 (22.4)	87 (26.1)
Age, y		
Mean (SD)	61.44 (10.20)	60.57 (10.20)
Median (IQR)	62.75 (55.18-68.91)	62.28 (54.58-67.25)
Weight, kg		
Mean (SD)	69.59 (11.82)	70.49 (12.02)
Median (IQR)	70.00 (60.00-76.00)	70.00 (62.00-79.00)
Time from onset to thrombolysis, h		
Mean (SD)	3.30 (0.86)	3.38 (0.89)
Median (IQR)	3.43 (2.70-4.03)	3.50 (2.75-4.13)
Stroke risk factor		
Hypertension	211 (63.9)	203 (61.0)
Diabetes	71 (21.5)	75 (22.5)
Hyperlipidemia	93 (28.2)	106 (31.8)
Heart disease[a]	65 (19.7)	64 (19.2)
Previous stroke >3 mo ago	87 (26.4)	84 (25.2)
Family history of stroke	17 (5.2)	25 (7.5)
Smoking	143 (43.3)	118 (35.4)
Heavy drinking	21 (6.4)	25 (7.5)
Blood pressure, mean (SD), mm HG		
Systolic	151.46 (22.08)	150.06 (21.17)
Diastolic	87.35 (12.70)	86.48 (12.62)
NIHSS score		
Mean (SD)	7.62 (3.71)	7.29 (3.40)
Median (IQR)	6.00 (5.00-10.00)	6.00 (5.00-9.00)

Abbreviations: NIHSS, National Institutes Health Stroke Scale; rhPro-UK, recombinant human prourokinase.

[a] Heart disease was defined as a range of major clinical heart and circulatory disease conditions, including but not limited to rhythm disorders, coronary artery disease, heart failure, and valvular disease.

Table 2. Primary and Secondary Outcomes

Outcome	Patients, No./total No. (%)		Risk difference (CI)[a]	P value[b]
	rhPro-UK group	Alteplase group		
Modified intention-to-treat analysis				
Primary (mRS score 0-1 at 90 d)	215/330 (65.2)	214/333 (64.3)	0.89 (−6.52 to 8.29)	.81
Secondary				
Major neurological improvement at 24 h[c]	132/330 (40.0)	142/333 (42.6)	−2.64 (−10.14 to 4.85)	.49
mRS score 0-2 at 90 d	251/330 (76.1)	250/333 (75.1)	0.99 (−5.56 to 7.53)	.77
Barthel Index score 75-100 at 90 d	272/330 (82.4)	271/333 (81.4)	1.04 (−4.82 to 6.90)	.73
Per-protocol analysis				
Primary (mRS score of 0-1 at 90 d	210/318 (66.0)	212/321 (66.0)	−0.01 (−7.48 to 7.47)	.99
Secondary				
Major neurological improvement at 24 h[c]	130/318 (40.9)	141/321 (43.9)	−3.04 (−10.70 to 4.62)	.44
mRS score 0-2 at 90 d	245/318 (77.0)	247/321 (76.9)	0.10 (−6.43 to 6.62)	.98
Barthel Index score 75-100 at 90 d	266/318 (83.6)	267/321 (83.2)	0.47 (−5.30 to 6.24)	.87

Abbreviations: mRS, modified Rankin Scale; rhPro-UK, recombinant human prourokinase.

[a] Primary outcome was analyzed using a CI of 95.4%, and secondary outcomes were analyzed using a CI of 95%.

[b] P values were tests to demonstrate superiority and were not tests to demonstrate noninferiority.

[c] Major neurological improvement was defined as a National Institutes of Health Stroke Scale score reduction of 4 or more or a score of 0 to 1.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

No significant difference between rhPro-UK and alteplase groups was observed using SITS-MOST (5 patients [1.5%] vs 6 patients [1.8%]; *P* > .99),[13] ECASS III (5 patients [1.5%] vs 6 patients [1.8%]); *P* > .99),[14] or NINDS (10 patients [3.0%] vs 7 patients [2.1%]; *P* = .47)[15] definitions of symptomatic ICH (**Table 3**). Systemic bleeding within 90 days occurred more frequently in the alteplase group (141 patients [42.2%]) than the rhPro-UK group (85 patients [25.8%]), a difference of 16.4 percentage points (*P* < .001). The occurrence of serious systemic bleeding was similar between rhPro-UK and alteplase groups (8 patients [2.4%] vs 9 patients [2.7%]; *P* > .99).

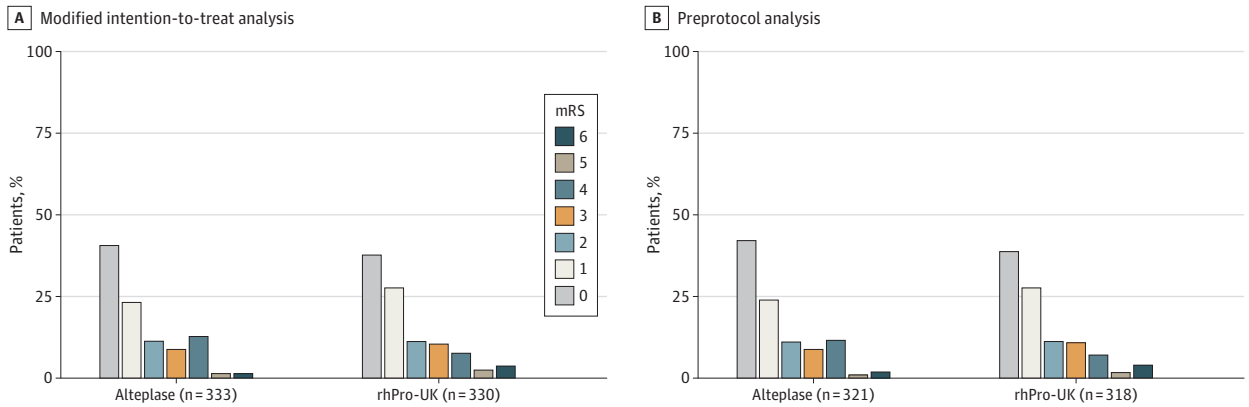
The percentage of patients showing recurrent ischemic stroke in the rhPro-UK group was similar to that in the alteplase group (2 patients [0.6%] vs 2 patients [0.6%]; *P* > .99). In the rhPro-UK group, 45 patients (13.6%) experienced at least 1 serious adverse event (SAE) in comparison with 44 patients (13.2%) in the alteplase group (*P* = .91). Groups showed no significant differences in the incidence of any serious adverse event.

Discussion

We conducted the PROST randomized clinical trial to gather large-scale prospective evidence of rhPro-UK efficacy in patients with AIS. In patients with AIS treated within 4.5 hours of onset, IVT with rhPro-UK was noninferior to treatment with alteplase (65.2% vs 64.3%; *P* = .81) and showed a 16.4 percentage point lower risk of systemic bleeding in comparison with alteplase treatment, which is consistent with data from the phase 2 trial.[11] Results of the secondary outcome analysis were also consistent and supported the effectiveness of rhPro-UK.

The dichotomous mRS score (0 or 1) was used as the primary outcome in this trial. A favorable functional outcome at 90 days was achieved in 65.2% of patients from the rhPro-UK arm and 64.3% of those from the alteplase group. Efficacy outcomes may be influenced by various factors, including the thrombolysis time window, age, and baseline stroke severity.[16] However, study groups showed no significant differences in baseline characteristics. In the Tenecteplase Versus Alteplase for Management of Acute Ischaemic Stroke (NOR-TEST) trial,[17] the thrombolytic agent was administered within 4.5 hours of stroke onset; 64% of patients in the tenecteplase group and 63% of those in the alteplase group achieved a favorable functional outcome at 90 days, which was similar to our results. The median NIHSS score at baseline in our trial was higher than that in the NOR-TEST trial (6 vs 4), but the mean age of patients in our study (61.44 years in the rhPro-UK group and 60.57 years in the alteplase group) was lower than that in the NOR-TEST trial (70.8 years in the tenecteplase group and 71.2 years in the alteplase group), and patients aged older than 80 years were not included in our trial. In a single-group clinical trial (Thrombolysis Implementation and Monitor of Acute Ischemic

Figure 2. Distribution of Modified Rankin Scale (mRS) Scores at 90 d



rhPro-UK indicates recombinant human prourokinase.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

Stroke in China [TIMS-China])[18] including patients treated with alteplase within 3 to 4.5 hours of AIS onset, 63.3% of patients achieved an mRS score of 0 to 1 at 90 days. The median NIHSS score of patients in the TIMS-China trial was 6, and the mean age was 61.1 years, similar to our study. The Non-Immunogenic Recombinant Staphylokinase Versus Alteplase for Patients With Acute Ischemic Stroke 4·5 h After Symptom Onset in Russia (FRIDA) trial[19] had a similar design as our study and involved the administration of staphylokinase or alteplase within 4.5 hours of stroke onset. But in comparison with our study, patients in the FRIDA trial had more severe neurological impairment at

Table 3. Safety Outcomes

Outcome	Patients, No. (%) (N = 664)		*P* value
	rhPro-UK group (n = 330)	Alteplase group (n = 334)	
All-cause death within 90 d	14 (4.2)	9 (2.7)	.30
From acute ischemic stroke	6 (1.8)	4 (1.2)	.54
From cardiovascular disease	1 (0.3)	0	.50
From intracranial hemorrhage	4 (1.2)	4 (1.2)	>.99
From other causes	3 (0.9)	1 (0.3)	.37
All-cause death within 7 d	9 (2.7)	4 (1.2)	.17
Symptomatic intracranial hemorrhage			
As defined in SITS-MOST[a]	5 (1.5)	6 (1.8)	>.99
As defined in ECASS III[b]	5 (1.5)	6 (1.8)	>.99
As defined in NINDS[c]	10 (3.0)	7 (2.1)	.47
Any intracranial hemorrhage within 90 d[d]	25 (7.6)	25 (7.5)	>.99
Systemic bleeding within 90 d	85 (25.8)	141 (42.2)	<.001
Gastrointestinal bleeding	29 (8.8)	90 (26.9)	<.001
Urological bleeding	39 (11.8)	38 (11.4)	.86
Mucocutaneous hemorrhage	4 (1.2)	5 (1.5)	>.99
Respiratory system bleeding	2 (0.6)	5 (1.5)	.45
Other	30 (9.1)	28 (8.4)	.75
Serious systemic bleeding within 90 d[e]	8 (2.4)	9 (2.7)	>.99
Gastrointestinal bleeding	0	1 (0.3)	>.99
Urological bleeding	0	0	NA
Mucocutaneous hemorrhage	0	0	NA
Respiratory system bleeding	1 (0.3)	0	.50
Other	7 (2.1)	8 (2.4)	>.99
Recurrent ischemic stroke within 7 d[f]	2 (0.6)	2 (0.6)	>.99
Serious adverse event within 90 d	45 (13.6)	44 (13.2)	.91

Abbreviations: ECASS, European Cooperative Acute Stroke Study; NA, not applicable; NINDS, National Institute of Neurological Disorders and Stroke; rhPro-UK, recombinant human prourokinase; SITS-MOST, Safe Implementation of Thrombolysis in Stroke-Monitoring Study.

[a] Symptomatic intracranial hemorrhage was defined according to the SITS–MOST and represented local or remote parenchymal hematoma type 2 on imaging performed within 36 hours after treatment, plus neurologic deterioration (as indicated by a National Institutes of Health Stroke Scale score ≥4 points compared with baseline or the lowest value between baseline and 24 hours) or hemorrhage leading to death.

[b] Symptomatic intracranial hemorrhage was defined according to ECASS III and represented any hemorrhage with neurologic deterioration (as indicated by a National Institutes of Health Stroke Scale score ≥4 points in comparison with baseline or the lowest value in the first 7 days) or any hemorrhage leading to death. In addition, the hemorrhage must have been identified as the predominant cause of neurologic deterioration.

[c] Symptomatic intracranial hemorrhage was defined according to the NINDS and represented any new hemorrhage associated with any neurologic deterioration.

[d] Any intracranial hemorrhage was defined as intracranial hemorrhage observed by computed tomography or magnetic resonance imaging after thrombolysis.

[e] The definition of serious systemic bleeding was any systemic bleeding reported as a serious adverse event.

[f] Recurrent ischemic stroke was defined as new stroke symptoms or new lesions on computed tomography or magnetic resonance imaging.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

baseline (median NIHSS score of 11 in both groups) and showed a lower proportion of favorable functional outcomes at 90 days (50% in the staphylokinase group and 41% in the alteplase group).

In our study, the incidence of systemic bleeding within 90 days in the rhPro-UK group (25.8%) was significantly lower than that in the alteplase group (42.2%). No significant difference in the rate of symptomatic ICH was observed between groups. Compared with the alteplase group, the rhPro-UK group had higher 3-month mortality, but the intergroup difference in the number of deaths was not significant. Each group had 5 deaths attributed to thrombolysis (1.5% vs 1.5%, $P > .99$), most of which were caused by fatal ICH. The remaining 13 deaths (9 deaths in the rhPro-UK group and 4 deaths in the alteplase group) were considered to be unrelated to thrombolysis. The death rate in this trial was similar to those reported in the NOR-TEST (5%)[17] and TIMS-China (5%) trials.[18] These findings suggest that rhPro-UK was safe in treating patients with AIS, which is in accordance with the safety profile shown in patients with AMI.

Our study found that the use of rhPro-UK 35 mg showed similar efficacy and safety as alteplase 0.9 mg/kg. There were several other advantages of rhPro-UK. Reduced risk of systemic bleeding in patients treated with rhPro-UK, indicating fewer complications, may mean lowered possibilities of use of blood products or hemostatic drugs, shorter hospital stays, and lower expenses. In developing countries, patient adherence to alteplase may decrease in large amounts owing to potential hemorrhagic complications, a high cost, and lower availability. Unlike alteplase, rhPro-UK does not need to be administered according to body weight, making it easier to administer and saving time (30 minutes vs 1 hour). Moreover, rhPro-UK is more cost-effective in most cases and is easily available in all levels of hospitals in China. These factors suggest that rhPro-UK may be a better option than alteplase for the management of AIS in developing countries.

Limitations

Our study has several limitations. First, the open-label design of this study may have introduced biases. However, we implemented multiple measures to minimize bias. For example, outcome-scoring investigators were trained and certified, and for end point assessment, a separate case report form of scales was provided to personnel who conducted analyses and efficacy evaluation so that they were blinded from the treatment information for each patient. On-site visits were performed in more than 80% of patients, and regular monitoring and auditing were implemented. The mITT and per-protocol analyses yielded similar results for the primary outcome, suggesting that these limitations were unlikely to have affected the primary outcome. Second, missing data for the mITT analysis were imputed by the last observation carried forward method. This may be a pessimistic assumption for each participant, but it may be not conservative for the comparison between trial groups. Thus, we conducted multiple imputation and analysis based on observed data for the primary outcome as a post hoc analysis. Given that the level of missing data was low, the methods showed similar results, suggesting that analysis results were robust. Third, advance imaging of perfusion lesion or vessel occlusion were not mandated. The information on reperfusion and recanalization on imaging was absent. However, the use of CT scans may give a wider applicability of this drug given that CT scans are widely available, even in smaller cities. More imaging evaluation will be conducted in further study. The safety and efficacy of rhPro-UK vs alteplase in patients receiving bridging therapy will be investigated in further study. Fourth, a 10% noninferiority margin is wider than the minimal clinically important difference that was defined in the 2005 Stroke Treatment Academic Industry Roundtable criteria and 2012 European Stroke Organisation outcomes working group consensus.[20-23] However, this choice was based on clinical and statistical reasoning from study investigators and clinical experts in China; retrospectively, this could have been better considered. Another phase 3 clinical trial with a larger sample size to identify the efficacy of rhPro-UK is in design.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

Conclusions

This randomized clinical trial found that administration of intravenous rhPro-UK within 4.5 hours of onset was noninferior to alteplase in patients with AIS. Compared with alteplase, rhPro-UK was associated with a similar risk of symptomatic ICH but a significantly lower risk of systemic bleeding. These findings require independent validation in the setting of another phase 3 randomized clinical trial.

ARTICLE INFORMATION

Accepted for Publication: May 31, 2023.

Published: July 25, 2023. doi:10.1001/jamanetworkopen.2023.25415

Open Access: This is an open access article distributed under the terms of the CC-BY-NC-ND License. © 2023 Song H et al. *JAMA Network Open*.

Corresponding Authors: Haiqing Song, MD, PhD (songhq@xwhosp.org), and Yuping Wang, MD, PhD (wangyuping@xwhosp.org), Department of Neurology, Xuanwu Hospital, Capital Medicine University, 45 Chang Chun St, Beijing 100053, China.

Author Affiliations: Department of Neurology, Xuanwu Hospital, Capital Medicine University, Beijing, China (Song, Yuan Wang, Ma, Yuping Wang); Department of Neurology, Duke University School of Medicine, Durham, North Carolina (Feng); Department of Clinical Medicine, Tasly Biopharmaceuticals Co, Ltd, Tianjin, China (R. Liu, Lv, Huang, Li); Department of Neurology, First Bethune Hospital of Jilin University, Changchun, Jilin, China (Yang); Department of Neurology, Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China (Geng); Department of Neurology, Harrison International Peace Hospital, Hengshui, Hebei, China (J. Zhu, Wei); Department of Neurology, General Hospital Northern Theater Command, Shenyang, Liaoning, China (Chen); Department of Neurology, Inner Mongolia Autonomous Region People's Hospital, Huhhot, Inner Mogolia, China (R. Zhu); Department of Neurology, Second People's Hospital of Huai'an, Huai'an, Jiangsu, China (Zhai); Department of Neurology, Xuzhou Central Hospital, Xuzhou, Jiangsu, China (Guo); Department of Neurology, First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, Inner Mongolia, China (B. Liu); Department of Neurology, Affiliated Hospital of Inner Mongolia Medical University, Huhhot, Inner Mogolia, China (Zhao).

Author Contributions: Drs Song and Yuping Wang had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Song and Yuan Wang contributed equally to this work and should share first authorship.

Concept and design: Song, Feng, Geng, Wei, Yuping Wang.

Acquisition, analysis, or interpretation of data: Yuan Wang, Ma, R. Liu, Lv, Huang, Li, Yang, Geng, J. Zhu, Wei, Chen, R. Zhu, Zhai, Guo, B. Liu, Zhao.

Drafting of the manuscript: Song, Yuan Wang, Feng, Li, Geng, Wei, Chen.

Critical review of the manuscript for important intellectual content: Ma, Feng, R. Liu, Lv, Huang, Li, Yang, Geng, J. Zhu, Wei, R. Zhu, Zhai, Guo, B. Liu, Zhao, Yuping Wang.

Statistical analysis: Li, Geng, Wei.

Obtained funding: Song, Wei, Yuping Wang.

Administrative, technical, or material support: Song, Yuan Wang, Ma, Yang, Wei, Zhai, Guo.

Supervision: Feng, R. Liu, Lv, Huang, Geng, J. Zhu, Wei, R. Zhu, B. Liu, Zhao.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was sponsored by Tasly Biopharmaceuticals Co, Ltd, who reimbursed for both alteplase and rhPro-UK.

Role of the Funder/Sponsor: The funder of the study (Tasly Biopharmaceuticals Co, Ltd) participated in the design, conduct, and supervision of the study and interpretation of data and reviewed and approved the manuscript. Authors who are employees of the company participated in each of these activities. The funder had no role in the collection, management, and analysis of the data; preparation of the manuscript; and decision to submit the manuscript for publication.

Group Information: The PROST collaborative group members are listed in Supplement 3.

Data Sharing Statement: See Supplement 4.

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

Additional Contributions: We would like to thank the patients and their families for participating in this trial and the members of the independent data monitoring committee (Mao-Lin He, MD [Beijing Shijitan Hospital]; Cui-Bai Wei, MD [Xuanwu Hospital, Capital Medical University]; and Gang Chen, PhD [Clinical Research Institute of Peking University), who judged the interim analysis results; we thank them for their advice. Tasly Biopharmaceuticals Co, Ltd reimburses contributors for conference-related transportation, accommodation, and meal costs. Data collection and statistical analysis were conducted by an independent statistical company, HBR Data Science Co, Ltd; Gaokui Zhang, PhD, was in charge of data collection and statistical analysis for the trial. The study sponsor paid the related labor costs for this work.

REFERENCES

1. Powers WJ, Rabinstein AA, Ackerson T, et al; American Heart Association Stroke Council. 2018 Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2018;49(3):e46-e110. doi:10.1161/STR.0000000000000158

2. Gurewich V. Experiences with pro-urokinase and potentiation of its fibrinolytic effect by urokinase and by tissue plasminogen activator. *J Am Coll Cardiol*. 1987;10(5)(suppl B):16B-21B. doi:10.1016/S0735-1097(87)80423-0

3. Suenson E, Lützen O, Thorsen S. Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. *Eur J Biochem*. 1984;140(3):513-522. doi:10.1111/j.1432-1033.1984.tb08132.x

4. Tomasi S, Sarmientos P, Giorda G, Gurewich V, Vercelli A. Mutant prourokinase with adjunctive C1-inhibitor is an effective and safer alternative to tPA in rat stroke. *PLoS One*. 2011;6(7):e21999. doi:10.1371/journal.pone.0021999

5. Pannell R, Kung W, Gurewich V. C1-inhibitor prevents non-specific plasminogen activation by a prourokinase mutant without impeding fibrin-specific fibrinolysis. *J Thromb Haemost*. 2007;5(5):1047-1054. doi:10.1111/j.1538-7836.2007.02453.x

6. Hao CH, Ding WX, Sun Q, et al. Effect of human recombinant prourokinase(rhpro-UK) on thromboembolic stroke in rats. *Eur J Pharmacol*. 2018;818:429-434. doi:10.1016/j.ejphar.2017.11.026

7. Hao C, Ding W, Xu X, et al. Effect of recombinant human prourokinase on thrombolysis in a rabbit model of thromboembolic stroke. *Biomed Rep*. 2018;8(1):77-84. doi:10.3892/br.2017.1013

8. Group PTS; PRIMI Trial Study Group. Randomised double-blind trial of recombinant pro-urokinase against streptokinase in acute myocardial infarction. *Lancet*. 1989;1(8643):863-868.

9. Vermeer F, Bösl I, Meyer J, et al; Practical Applications of Saruplase Study. Saruplase is a safe and effective thrombolytic agent; observations in 1,698 patients: results of the PASS study. *J Thromb Thrombolysis*. 1999;8(2):143-150. doi:10.1023/A:1008967219698

10. Bär FW, Meyer J, Vermeer F, et al; SESAM Study Group. The Study in Europe with Saruplase and Alteplase in Myocardial Infarction. Comparison of saruplase and alteplase in acute myocardial infarction. *Am J Cardiol*. 1997;79(6):727-732. doi:10.1016/S0002-9149(97)89274-0

11. Song H, Wang Y, Ma Q, et al. Efficacy and safety of recombinant human prourokinase in acute ischemic stroke: a phase IIa randomized clinical trial. *Transl Stroke Res*. 2022;13(6):995-1004. doi:10.1007/s12975-022-01012-9

12. Demaerschalk BM, Kleindorfer DO, Adeoye OM, et al; American Heart Association Stroke Council and Council on Epidemiology and Prevention. Scientific rationale for the inclusion and exclusion criteria for intravenous alteplase in acute ischemic stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2016;47(2):581-641. doi:10.1161/STR.0000000000000086

13. Wahlgren N, Ahmed N, Dávalos A, et al; SITS-MOST investigators. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. 2007;369(9558):275-282. doi:10.1016/S0140-6736(07)60149-4

14. Hacke W, Kaste M, Bluhmki E, et al; ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. 2008;359(13):1317-1329. doi:10.1056/NEJMoa0804656

15. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333(24):1581-1587. doi:10.1056/NEJM199512143332401

16. Wahlgren N, Ahmed N, Dávalos A, et al; SITS investigators. Thrombolysis with alteplase 3-4.5 h after acute ischaemic stroke (SITS-ISTR): an observational study. *Lancet*. 2008;372(9646):1303-1309. doi:10.1016/S0140-6736(08)61339-2

17. Logallo N, Novotny V, Assmus J, et al. Tenecteplase Versus Alteplase for Management of Acute Ischaemic Stroke (NOR-TEST): a phase 3, randomised, open-label, blinded endpoint trial. *Lancet Neurol*. 2017;16(10):781-788. doi:10.1016/S1474-4422(17)30253-3

18. Zheng H, Yang Y, Chen H, et al. Thrombolysis with alteplase 3-4.5 hours after acute ischaemic stroke: the first multicentre, phase III trial in China. *Stroke Vasc Neurol*. 2020;5(3):285-290. doi:10.1136/svn-2020-000337

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023

19. Gusev EI, Martynov MY, Nikonov AA, et al; FRIDA Study Group. Non-immunogenic recombinant staphylokinase versus alteplase for patients with acute ischaemic stroke 4·5 h after symptom onset in Russia (FRIDA): a randomised, open label, multicentre, parallel-group, non-inferiority trial. *Lancet Neurol*. 2021;20(9):721-728. doi:10.1016/S1474-4422(21)00210-6

20. Albers GW, Goldstein LB, Hess DC, et al; STAIR VII Consortium. Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. *Stroke*. 2011;42(9):2645-2650. doi:10.1161/STROKEAHA.111.618850

21. Saver JL, Mistry E. Time to recognize three classes of non-inferiority trial margins. *J Neurointerv Surg*. 2023;15(1):2-4. doi:10.1136/neurintsurg-2022-018879

22. Bath PM, Lees KR, Schellinger PD, et al; European Stroke Organisation Outcomes Working Group. Statistical analysis of the primary outcome in acute stroke trials. *Stroke*. 2012;43(4):1171-1178. doi:10.1161/STROKEAHA.111.641456

23. Cranston JS, Kaplan BD, Saver JL. Minimal clinically important difference for safe and simple novel acute ischemic stroke therapies. *Stroke*. 2017;48(11):2946-2951. doi:10.1161/STROKEAHA.117.017496

SUPPLEMENT 1.

Trial Protocol and Statistical Analysis Plan

SUPPLEMENT 2.

eTable 1. Reasons for Patients Who Did Not Complete the 90-d Visit
eTable 2. Distribution of Modified Rankin Scale Scores at 90 d
eTable 3. Primary Outcome Using Multiple Imputation
eTable 4. Primary Outcome Based on Only Observed Data
eFigure. Subgroup Analysis of Favorable Outcome at Day 90 by Demographic Characteristics and Baseline Clinical Data

SUPPLEMENT 3.

Nonauthor Collaborators. PROST collaborative group

SUPPLEMENT 4.

Data Sharing Statement

Downloaded From: https://jamanetwork.com/ Poria Medical Center by Eran Or on 08/13/2023