Appendix A: Supplementary Methods

Appendix A1. Analysis Details

This analysis includes all trials that were identified for a systematic review of studies looking at recurrent stroke with patent foramen ovale (PFO). The SCOPE PI (Kent) was part of the team that performed this systematic review, which was updated in August 2019 for guideline development by the American Academy of Neurology (AAN). And subsequently updated to September 2021 for this article. Based on this systematic search performed of Medline and Embase, these studies represent the *totality of available randomized evidence on the use of percutaneous implanted devices for PFO closure versus medical therapy in patients with PFO-associated cerebral ischemic events*. Complete information about the search strategy and systematic review can be found in the original guidance.[1] **Appendix B1** shows a PRISMA flowchart of all studies identified.

All RCTs identified in the systematic review provided individual patient-level study data. Data entered into the central SCOPE database were a limited dataset (LDS), with all high-level patient identifiers removed. All data were collected under the aegis and supervision of the SCOPE Steering Committee, and integrated and stored at the Predictive Analytics and Comparative Effectiveness (PACE) Center at Tufts Medical Center, Boston, MA. The data were harmonized and analyzed by two statisticians at the PACE Center, Tufts Medical Center to ensure they accurately matched the values reported by the trials. **Appendix A5** describes variables that were harmonized, including ASA and shunt size. There were no issues identified in checking IPD.

The PI of this study (Kent) developed an initial list of variables based on variables used in a prior 3-trial individual patient meta-analysis[2] and variables that make up the Risk of Paradoxical Embolism (RoPE) Score[3,4]. The list was further expanded and refined at an investigator meeting in February 2020. **Appendix Table 1** displays the variables collected.

Appendix B4 provides the patient-level characteristics for each study, and note where data was missing.

All analyses were conducted using SAS (Version 9.4) and R (Version 4.0.2).

Examination of proportional hazards assumption

Proportional hazards assumptions were assessed using graphical and statistical test-based methods. Visual assessment of the log-log survival curve for each treatment group in each trial was used to detect violations of proportionality. Time-dependent covariates — interactions between the predictors and log(time) — were included to assess proportionality for each predictor. Additionally, tests of proportional hazards assumption was based on scaled Schoenfeld residuals for each predictor and overall (global test).[5] No visual or statistical violation of proportional hazards was observed.

Handling of missing data

Missing values for covariates were imputed using fully conditional specification methods (predictive mean matching for continuous variables and discriminant function method for all dichotomous variables) to generate 10 complete data sets.[6] The imputation model for each variable with missing values included all pre-specified covariates and the outcome. Analyses were conducted in each of the 10 compete data sets separately and pooled using Rubin's Rules.

Random effects Cox proportional hazards regression

Study-specific random effects were modeled using SAS PROC PHREG procedure using the RANDOM statement to fit a shared frailty model for clustered data.[7] The log-normal distribution of shared frailty was used and the common variance parameter (covariance estimate = 0.13; asymptotic standard error = 0.12) was estimated using residual maximum likelihood.

Assessment of linear assumption

The functional form of continuous variables (age and RoPE Score) was assessed for linearity using higher order polynomial terms (i.e., quadratic). These higher order terms were tested for statistical significance and model fit was assessed by differences in likelihood ratio compared to models with a linear relationship. We found no evidence of statistically significant non-linear associations with the treatment effect.

Appendix Table 1. Variables of Interest.

Category	Variable
Clinical Variables	Age (at time of stroke)
	Sex
	Coronary artery disease
	Diabetes
	Hypertension
	Hyperlipidemia
	Prior spells: number, date(s), event(s)
	Smoking status: current
	Body Mass Index
	Index event: stroke or TIA
	Index event: date
	Medication at index event: statin, antiplatelet, anticoagulant, CP/HRT
Echocardiographic Variables	Mobility of septum: normal, hypermobile
	PFO size: large, not large
	Shunt at rest: yes, no
Neuroradiology Variables	Index stroke seen: yes, no

	Location: superficial, deep
	Size: large, small/not seen
	Multiple: yes, no (not seen = single)
	Prior stroke: yes, no
Treatment Variables	Warfarin (anticoagulant, Coumadin)
	Antiplatelets
Follow-Up Variables	Date of last follow-up
	Duration of follow-up
	Recurrent stroke
	Recurrent TIA
	Date of recurrent event
	Death
	Date of death
	Cause of death
	PFO closure (treatment)
	Atrial Fibrillation, all and after 45 days (safety)
	Major Bleeding (safety)
	Procedural complication (safety)
Cohort Designation and Randomization	Intent-to-treat group (closure vs. medical therapy)
	Per-protocol group (closure vs. medical therapy vs. excluded)
	As-treated group (closure vs. medical therapy vs. excluded)

CP, contraceptive pill; HRT, hormone replacement therapy; PFO, patent foramen ovale; TIA indicates transient ischemic attack.

Appendix A2. RoPE Score Detail

Patent foramen ovale (PFO) are randomly distributed in the general population in about 25% of adults, and not associated with other vascular risk factors. However, among patients with cryptogenic stroke (CS), the presence of a PFO is highly associated with the absence of conventional vascular risk factors and the presence of specific neuroimaging findings (a superficial cortical infarct). This negative association arises from index event (or "collider") bias;[8] that is, it is induced because vascular risk factors and PFO are causes of the same outcome (i.e., cryptogenic stroke).

Based on this observation, we developed a model to predict the presence of PFO in patients with otherwise cryptogenic stroke and transformed this probability, using Bayes Theorem, into a "patient-specific" attributable fraction — i.e., the fraction of cryptogenic strokes that are attributable to PFO in a group of patients sharing a Risk of Paradoxical Embolism (RoPE) Score, according to the following equation:

$$\text{PFO Attributable Fraction} = 1 - \left(\frac{\text{Prevalence of PFO in controls} \times [1 - \text{Prevalence of PFO in CS cases}]}{\text{Prevalence of PFO in CS cases} \times [1 - \text{Prevalence of PFO in controls}]}\right)$$

We found that easily obtainable clinical characteristics can identify CS patients who vary markedly in the prevalence of PFO, reflecting substantial and clinically important variation in the probability that a discovered PFO is likely to be causally related to the stroke rather than an incidental present (**Appendix Table 2**). For example, a PFO is discovered in just 23% of cryptogenic stroke patients in the lowest RoPE Score strata, which is approximately the same as the general population—indicating that PFOs in these patients are almost always an incidental finding. Conversely, PFOs are found in greater than 70% of cryptogenic stroke patients with a RoPE Score of 9-10, indicating almost a 90% probability that the stroke can be attributed to the presence of the PFO.

Appendix Table 2. PFO-Attributable Fraction by RoPE Score.[4] Cryptogenic stroke n=3023.

RoPE Score	Patients, N (n=3023)	Prevalence of PFO % (95% CI)	PFO-Attributable Fraction[a] % (95% CI)	Estimated 2-yr stroke/TIA recurrence rate (among those with PFO, n=1324)[4]
0-3	613	23% (19% to 26%)	0% (0% to 4%)	20 (12-28)
4	511	35% (31% to 39%)	38% (25% to 48%)	12 (6-18)
5	516	34% (30% to 38%)	34% (21% to 45%)	7 (3-11)
6	482	47% (42% to 51%)	62% (54% to 68%)	8 (4-12)
7	434	54% (49% to 59%)	72% (66% to 76%)	6 (2-10)
8	287	67% (62% to 73%)	84% (79% to 87%)	6 (2-10)
9-10	180	73% (66% to 79%)	88% (83% to 91%)	2 (0-4)

[a]Based on the observed prevalence of PFO, rather than the predicted, and assumes a population prevalence of PFO of 25%.
CI, confidence interval; PFO indicates patent foramen ovale; TIA, transient ischemic attack.

The RoPE Score has been externally validated by independent teams to predict the presence of a PFO in the CS population[9,10] and it is widely used in shared decision making. However, it is not intended to be used in isolation. The premise of the RoPE Study was that mechanical closure will benefit patients with a high *attributable recurrence risk*, which can be thought of as the product of the attributable fraction (predicted by the RoPE Score) and the stroke recurrence risk. A higher RoPE Score, however, is associated with a lower recurrence risk. In the RoPE study the 2 year risk of stroke/transient ischemic attack (TIA) recurrence of patients with a RoPE Score of 0 to 3 was ~20 but was only ~2% in those with a RoPE Score of 9 to 10.[4]

Further, the methods used to develop the RoPE Score (prediction of the presence of a PFO in cryptogenic stroke patients) did not permit high risk anatomic features of the PFO itself (such as the size of the left-to-right shunt and the presence of an atrial septal aneurysm) to be incorporated into

the Score. For these reasons, recent consensus documents suggest that the RoPE Score should be part of a broader evaluation to help determine those patients whose PFO is most likely to be caused by a PFO-related mechanism who might benefit from closure.[11-13]

Appendix A3. PASCAL Score Detail

To further improve the identification of ischemic strokes due to patent foramen ovale, an international consensus group recently proposed the PFO-Associated Stroke Causal Likelihood (PASCAL) Classification System (**Appendix Figure 1**). This is different from the other three and directly germane to the current study. Among patients with no major defined cause of ischemic stroke, the PASCAL classification system integrates information regarding: 1) presence of features that increase likelihood of PFO-stroke mechanisms (high risk PFO physiologic and structural features of large shunt or atrial septal aneurysm), and 2) absence of features that increase likelihood of an occult non-PFO stroke mechanisms (older age, vascular risk factors, and stroke topography features) as quantified in the RoPE score. Based on this combination of factors, the original, extended PASCAL Classification System algorithmically assigns a likelihood of causal relationship among five levels: Definite, Highly Probable, Probable, Possible, and Unlikely.[16] The PASCAL algorithm was developed using a mixed methods approach incorporating expert judgement, physiologic and epidemiologic data, and the validated RoPE Score. The original, extended PASCAL Classification system is shown in **Appendix Figure 1**.

Appendix Figure 1. The Extended PFO-Associated Stroke Causal Likelihood (PASCAL) Classification System.

Risk Grade	Features	Casual Relatedness	
		Low RoPE Score[a]	High RoPE Score[a]
Very high risk	PFO + straddling thrombus	Definite	Definite
High risk	BOTH of: 1A. PFO + ASA, *or* 1B. Large shunt PFO, *AND* 2. PE or DVT preceding index infarct	Probable	Highly Probable
Medium risk	ANY of: 1. PFO + ASA 2. Large shunt PFO	Possible	Probable
Low risk	Small shunt PFO without ASA	Unlikely	Possible

[a]The RoPE Score includes points for 5 age categories, cortical infarct, absence of hypertension, diabetes, prior stroke or transient ischemic attack, and smoking. A higher RoPE score (≥ 7 points) increases probability of causal association.
ASA, atrial septal aneurysm; DVT, deep vein thrombosis; PE, pulmonary embolism; PFO indicates patent foramen ovale; RoPE, Risk of Paradoxical Embolism.

While data regarding many of the patient features used in the extended PASCAL Classification system were collected in the RCTs analyzed in the SCOPE project, two were not: 1) the presence of a thrombus straddling the PFO opening (supporting Definite causal relatedness), and 2) the occurrence of a PE or DVT shortly before or concurrent with the index ischemic stroke (supporting Highly Probable or Probable causal relatedness). Accordingly, for the current pooled analysis a simpler PASCAL classification system was developed by censoring those two uncollected patients' features and using the collected patient features to algorithmically assign patients to three levels of likelihood of causal relationship: Probable, Possible, and Unlikely (main manuscript Table 1B). The SCOPE protocol prespecified as one of its primary aims testing for heterogeneity of treatment effect in the pooled RCT data based on patient PASCAL Probable, Possible, and Unlikely grades.

Appendix A4. Definitions of "Per-protocol" and "As-treated" Populations

Systematic, Collaborative, PFO closure Evaluation (SCOPE)	**Per-Protocol population (if possible to identify across trials): all patients who: i) received the randomly assigned treatment, ii) adhered at least moderately to the trial-mandated long-term medical treatment specific to their allocated treatment group (including long-term antithrombotic therapy in the medical therapy-only treatment group and long-term post-device antithrombotic therapy in the closure device plus medical therapy group, iii) did not have a major inclusion or exclusion violation, classified according to the treatment group to which they were randomly assigned and iv) patients who are NOT lost to follow up, when these patients are able to be identified (special considerations for PC and RESPECT trials)**
CLOSE	*An additional analysis was performed in the per-protocol cohort, which included patients who received the randomly assigned treatment, adhered to the protocol-mandated medical treatment **until the end of the trial**, and did not have a major protocol violation.*
PC Trial	*In a per-protocol analysis, we restricted the analysis to data from patients in the closure group in whom implantation of a device was attempted and patients in the medical-therapy group who received treatment as assigned at the time of randomization; if patients in the medical-therapy group crossed over to the closure group, the data were censored at the time of crossover.* **Special consideration:** • PC Trial censored people who crossed over at the time of crossover in their PP analysis. We decided we would not do this, and instead exclude patients who crossed over. • In their publication, they used the **LTFU at 3 years** to identify and report. Using the 3 year variable would hopefully be consistent with their publication and make their definition closer to the other trials.
CLOSURE	*Defined as all randomized patients who received the treatment to which they were randomized, who had no major inclusion/exclusion criteria violations, and **who had a follow-up of at least 22 months.***
RESPECT	*The per-protocol cohort included patients who received the randomly assigned treatment, adhered to the protocol-mandated medical treatment, and did not have a major inclusion or exclusion violation.* **Special consideration:** • Respect did not exclude patients who were lost to follow up in their per protocol analysis. In their short-term publication, they identified *119* patients who "discontinued prior to primary endpoint", and in their long-term follow-up publication, they identified *264* patients who "discontinued prior to primary endpoint." • In the data they provided, they provided information about *226* patients who discontinued, these patients have been excluded from the SCOPE per-protocol analysis.

REDUCE	*For per-protocol (PP) analysis, only subjects who were randomized and treated according to critical protocol requirements were analyzed, according to treatment assigned at randomization. Specifically, subjects randomized to the closure group who received antiplatelet medical therapy and PFO closure with a study device within 90 days post-randomization, and subjects randomized to medical therapy who received antiplatelet medical therapy and no PFO closure by any means at any time, were included in the PP analysis. The PP population excludes subjects who violated key eligibility criteria, did not receive the therapy to which they were randomized, or did not comply with one of the protocol required medical regimens.*
DEFENSE	*Included patients who received the randomly assigned treatment, adhered to the protocol-mandated medical treatment until the end of the trial, and did not have a major protocol violation.*

SCOPE "As treated" population definition:

All the patients in the study classified according to the treatment actually received (i.e., this analysis will compare patients who "got device" versus those that did not). Patients randomized to medication but got device are censored at time of crossover to the device arm.

Special consideration: PC trial did not provide device procedure dates for all patients.

Appendix A5. Description of Atrial Septal Aneurysm and Shunt Size Variables

Appendix Table 3. Variable Definition for ASA Class.

SCOPE Excursion Class	Systematic, Collaborative, PFO closure Evaluation (SCOPE)	defined as ≥10 mm of excursion from midline
TOTAL	CLOSURE	*mobility of septum of 10 mm or greater total excursion of the septum*
midline	PC Trial	*protrusion of the interatrial septum, or part of it, of more or equal to 15mm beyond the plane of the interatrial septum and the diameter of the aneurysm base measured at least 15mm.*
TOTAL	RESPECT	*defined as ≥10 mm septum primum excursion*
TOTAL	REDUCE	*defined as the movement of the septum primum into either atrium for a total excursion of at least 10 mm (from an imaginary midline).*
midline	DEFENSE	*ASA based on Defense defined asa or hypermobile septum, where ASA=atrial septal aneurysm (protrusion of the dilated segment of the septum at least 15 mm beyond the level surface of the atrial septum), hypermobility (phasic septal excursion into either atrium ≥10 mm)*
TOTAL	CLOSE	septum primum excursion greater than 10mm as identified on TEE

PFO indicates patent foramen ovale; TEE, transesophageal echocardiogram.

Appendix Table 4. Variable Definition for Large Shunt Size.

Systematic, Collaborative, PFO closure Evaluation (SCOPE)	Target: Large shunt size was defined in our database as >20+ bubbles (values below in BLUE coded as 'large' in our database)
CLOSURE	*Small: (1) None; (2): Trace, 1~10 bubbles, (3) Moderate, ~10-25 bubbles*, ***Large: (4) Substantial, ~25 or more bubbles***

PC Trial	*Small: grade 0 = none; grade 1 = minimal (1-5 bubbles), grade 2 = moderate (6 to 20 bubbles),* ***Large: grade 3 = severe (>20 bubbles)***
RESPECT	*Small: Grade 0 (none), Grade 1 = 1-9 bubbles; Grade 2 = 10 to 20 bubbles;* ***Large: Grade 3 = over 20 bubbles***
REDUCE	*PENN RE-READ FROM TEE (IF MISSING (~20% of time), USED ORIGINAL DATA FROM GORE):* *Small :(0)Grade 0[no bubbles], (1)Grade 1 [1-9 bubbles], (2)Grade 2 [10-20] bubbles,* ***Large: (3)Grade3 [>20 bubbles]***
DEFENSE	*Small: (≤20 Microbubbles),* ***Large (>20 microbubbles)***
CLOSE	*Small : ≤30 Bubbles on TTE or TEE,* ***Large: >30 microbubbles on TTE or TEE***

PFO indicates patent foramen ovale; TEE, transesophageal echocardiogram; TTE, transthoracic echocardiogram;