

**CONCISE REVIEW**

# Human endothelial colony-forming cells in regenerative therapy: A systematic review of controlled preclinical animal studies

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**Abstract**

Endothelial colony-forming cells (ECFCs) hold significant promise as candidates for regenerative therapy of vascular injury. Existing studies remain largely preclinical and exhibit marked design heterogeneity. A systematic review of controlled preclinical trials of human ECFCs is needed to guide future study design and to accelerate clinical translation. A systematic search of Medline and EMBASE on 1 April 2019 returned 3131 unique entries of which 66 fulfilled the inclusion criteria. Most studies used ECFCs derived from umbilical cord or adult peripheral blood. Studies used genetically modified immunodeficient mice ( $n = 52$ ) and/or rats ( $n = 16$ ). ECFC phenotypes were inconsistently characterized. While  $>90\%$  of studies used CD31+ and CD45–, CD14 – was demonstrated in 73% of studies, CD146+ in 42%, and CD10+ in 35%. Most disease models invoked ischemia. Peripheral vascular ischemia ( $n = 29$ ), central nervous system ischemia ( $n = 14$ ), connective tissue injury ( $n = 10$ ), and cardiovascular ischemia and reperfusion injury ( $n = 7$ ) were studied most commonly. Studies showed predominantly positive results; only 13 studies reported  $\geq 1$  outcome with null results, three reported only null results, and one reported harm. Quality assessment with SYRCLE revealed potential sources of bias in most studies. Preclinical ECFC studies are associated with benefit across several ischemic conditions in animal models, although combining results is limited by marked heterogeneity in study design. In particular, characterization of ECFCs varied and aspects of reporting introduced risk of bias in most studies. More studies with greater focus on standardized cell characterization and consistency of the disease model are needed.

**KEYWORDS**

controlled studies, ECFCs, preclinical, regenerative medicine, systematic review

**1 | INTRODUCTION**

Endothelial colony forming cells (ECFCs) have with robust proliferative potential and self-renewal capacity, making them of particular

interest for cell-based regenerative therapy of diseases with a vascular etiology.<sup>1,2</sup> Animal models of ischemic peripheral vascular injury, cerebral ischemia, and ischemic retinopathy have been used to study ECFC-based treatments.<sup>3</sup> ECFCs have been researched under various

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additional monikers, including but not limited to, “blood outgrowth endothelial cells” and “late outgrowth endothelial cells.”<sup>4-7</sup> Ambiguity, however, persists regarding the reported characterization of ECFCs used in some preclinical studies. More recent stringent criteria proposed by Medina et al (henceforth referred to as the Medina Criteria) offer a more precise and a reproducible definition of cell type based on a profile of cell surface expression markers and allows for the comparison between studies and appropriate pooling of results from different studies to assess the efficacy of ECFC-based therapy.<sup>8</sup> A systematic review of the literature is needed to evaluate preclinical studies using ECFCs in animal models of disease to assess cell characterization methods and other aspects of study design and treatment administration that are associated with potential benefit. This will accelerate the development of more definitive preclinical studies and will propel the field of ECFC therapy toward informative clinical trial development.

## 2 | METHODS

### 2.1 | Systematic search and study selection

A detailed description of the registered protocol for our systematic review is available on PROSPERO (Record ID: CRD42019140115) and was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting systematic reviews.<sup>9</sup> In brief, a systematic search for keywords relating to vascular progenitors and ECFCs was conducted on Medline and EMBASE without limits on year or language of publication up to 1 April 2019. Our search was designed to capture a broad range of articles that used any type of endothelial progenitor cell to treat or repair end organ or organ system damage in preclinical animal models of disease. The title and abstract of each record were screened in duplicate using Covidence Systematic Review Software to identify publications with potential relevance. Conflicts were resolved by reaching consensus between two reviewers (G. L., K. Z.) and/or involving a third reviewer (D. S. A.). Initial (level 1) screening of selected studies was conducted using predetermined criteria and was aimed at excluding studies which lacked control groups, did not use a cell type that was closely or potentially related the definition of ECFC as outlined by the Medina Criteria,<sup>8</sup> or did not study ECFCs in an animal model of disease (ie, excluded *in vitro* studies and studies where

### Significance statement

This review summarizes past progress, the present state, and the future potential of endothelial colony-forming cell therapy—stem cell therapy for vascular repair. Studies identified through a systematic search are discussed, focusing on preclinical data from animal studies, highlighting both the drawbacks and the novel findings leading to the progress of stem cell therapies into the clinic.

cells or cell-derived products were not administered to animals). Full-text eligibility determination (level 2 screening), was conducted by exporting all selected citations to EndNote X9 (Clarivate Analytics, Toronto, Canada), including full-text PDFs and their accompanying supplements. Full texts that could not be retrieved automatically were manually collected and uploaded. Full texts were assessed for inclusion and exclusion criterion in duplicate by two separate reviewers (G. L., K. Z.) and any conflicts were resolved by discussion and/or a third reviewer (D. S. A.). At this stage, criteria ensured that the cells used in the studies were of human origin and were characterized sufficiently to be considered ECFCs. Sufficient characterization specifically meant adequate documentation of cell surface marker expression using modified, or minimal, criteria from the aforementioned Medina Criteria (Figure 1). Sufficient reporting of ECFC characterization included information within the primary article, within a referenced protocol that included ECFC characterization, or within a previously published study that provided adequate reporting of ECFCs. All studies had to include adequate controls and report on at least one outcome related to the organ injury or function. More detail regarding the specific inclusion and exclusion criteria can be found in the published protocol on PROSPERO.

### 2.2 | Determining risk of bias, data extraction, and data analysis

Risk of bias analysis of study methods and reporting was conducted in duplicate using SYRCLE which is derived from the Cochrane risk of bias tool and has been adapted to preclinical animal trials.<sup>10</sup> Data extraction was done in duplicate and collected using Airtable (Airtable, San Francisco, California), and included study characteristics, including

Full Medina Criteria	Modified Medina Criteria
All: CD31+, CD105+, and CD146+	At least one: CD31+, CD105+, CD146+; None: CD31–, CD105–, CD146–
Both: CD45– and CD14–	At least one: CD45–, CD14–; Neither: CD45+, CD14+
<b>Both Criteria Require</b> Intrinsic tube forming capacity <i>in vitro</i> and <i>in vivo</i>	

**FIGURE 1** Complete Medina Criteria compared to the modified Medina Criteria. Figure adapted from the Medina et al study<sup>8</sup>

the species of animals used, specifics of the disease model studied, cell characterization information, reported outcomes, and whether or not benefit was reported. Data concerning the Medina criteria for ECFC characterization was extracted and studies were categorized accordingly.

### 3 | RESULTS

#### 3.1 | Characteristics of included studies

A total of 3142 studies were identified in our systematic search. Eleven duplicates were automatically removed, and 2689 records were excluded after screening for potential relevance by title and abstract. A total 442 full texts were assessed for eligibility, and 376 were excluded: 130 records were abstracts only, 116 records used cells that were not human, 46 records did not provide details of cell characterization, 20 were recognized as additional duplicate publications, 18 publications were not available in English, 15 publications did not study ECFCs, 13 studies lacked appropriate controls, 13 studies did not administer cell products with therapeutic intent, and 5 studies administered cells to humans. A total of 66 studies were included in our final review and analysis (Figure 2). All studies that were included were published in 2009 or later. Publication rates peaked in 2014 with 10 studies. The largest number of studies were

published by investigators from France, Korea, China, and Canada (Supporting Information 1).

All included studies used either mice or rats in their animal models (Table 1). Multiple genetic strains of mice were used, with the most common being various immunodeficient subtypes (BALB/c, NOD-SCID) followed by C57BL/6J. Rats were predominantly Sprague Dawley. No other species of animals were used. ECFCs were derived predominantly from human umbilical cord blood ( $n = 55$ ; 83%) while remaining studies expanded ECFCs from peripheral blood ( $n = 11$ ; 17%) of healthy adult volunteers.<sup>11-21</sup> One study used ECFCs collected from human placenta.<sup>22</sup>

Most ( $n = 59$ , 89%) studies administered ECFCs while 12 studies also included experiments with derivative products such as extracellular vesicles (ie, exosomes<sup>23-27</sup> or microvesicles<sup>11,28</sup>), and/or conditioned media<sup>7,29-32</sup> (Table 1). The majority of studies administered cells and/or derivative products via intravenous injection ( $n = 32$ ; 48%) or intramuscularly ( $n = 15$ ; 23%). A single study explored topical

**TABLE 1** Study characteristics

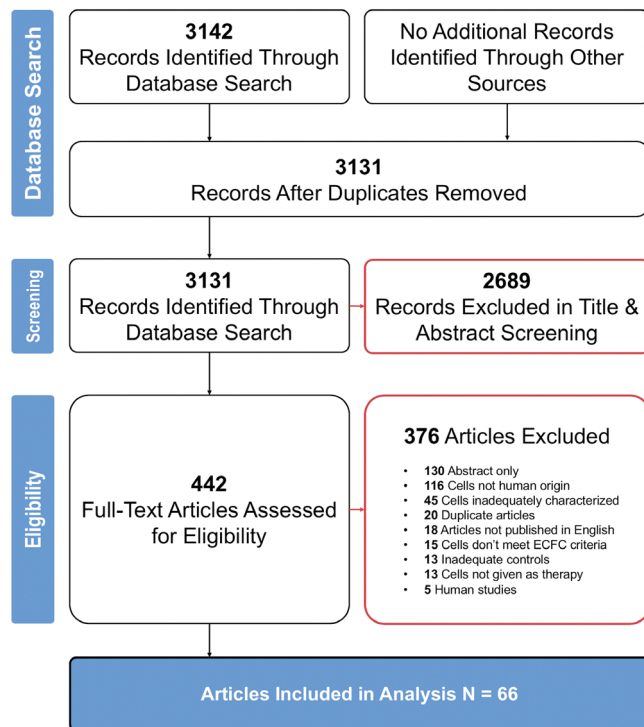
Study characteristic	# of studies, n (%) (total n = 66)
<b>Animal species used</b>	
Mouse	50 (76)
Rat	14 (21)
Both	2 (3)
<b>ECFC source</b>	
Umbilical cord	55 (83)
Peripheral	11 (17)
Placental	1 (2)
<b>Interventions studied</b>	
ECFC (cells only)	54 (82)
ECFC conditioned media	5 (8)
ECFC extracellular vesicles	7 (11)
<b>Route of administration</b>	
Systemic—33 (50)	
Intravenous or arterial	33 (50)
Local—36 (54) <sup>a</sup>	
Intramuscular	15 (23)
Intramyocardial	4 (6)
Intravitreal	4 (6)
Intraperitoneal	3 (5)
With transplanted tissue	3 (5)
Other <sup>b</sup>	12 (18)

Note: In some cases, studies reported more than one category of characteristic listed below, and the total numbers may add up to more than 100%.

<sup>a</sup>Three articles assess local administration also assessed systemic administration.

<sup>b</sup>Other includes: intracavernous (two studies), retro-orbital injection (two studies), subcutaneous (two studies) and intra-aortal, intradermal, intrathecal, intraventricular, subcapsular injection, and topical (one study each).

Abbreviation: ECFC, endothelial colony-forming cell.



**FIGURE 2** PRISMA diagram outlining the screening process. Specific reasons for exclusion are included for the full-text assessment

application as a method of delivering either cells and/or cell-derived products.<sup>31</sup>

### 3.2 | Risk of bias

The risk of potential bias in the studies included in our analysis was assessed with the SYRCL tool and reveals an overall high or unclear risk of bias. Most studies controlled for baseline characteristics between experimental groups well and most studies were not affected by “other sources of bias” such as nonpublic funding sources and conflicts of interest. However, many studies did not describe allocation concealment, blinding of investigators to the intervention, and random outcome assessment (see Table 2). Selective outcome reporting was unclear across the board as there were no readily available a priori protocols that allowed for the assessment of whether or not the data and results reported were congruent with the intention of the study at its inception.

### 3.3 | Cell and cell product characterization

As part of the inclusion criteria, all studies fulfilled our modified Medina criteria (Figure 1). Reporting of cell surface markers was heterogeneous (Figure 3). In general, a large majority of studies reported CD31 as a positive marker (n = 61; 92%) and CD45 as a negative marker (n = 62; 94%). Absence of CD14 was reported in most (n = 48; 73%) studies as a negative marker. Positive surface expression of CD105 and CD146 were reported in 35% and 42% of studies, respectively. Only 11 studies (17%) met the complete Medina criteria,<sup>18,19,29,33-40</sup> with just four studies (6%)<sup>19,29,34,38</sup> reporting complete characterization information within the primary article (Supporting Information 2).

**TABLE 2** Risk of bias using criteria from the SYRCL risk of bias tool

Risk domain	Low	Unclear	High
Sequence generation	3%	33%	64%
Baseline characteristics	77%	18%	5%
Allocation concealment	3%	2%	95%
Random housing	0%	33%	67%
Blinding (intervention)	3%	2%	95%
Random outcome assessment	12%	11%	77%
Blinding (outcome assessment)	42%	5%	53%
Incomplete outcome data	39%	59%	2%
Selective outcome reporting	0%	100%	0%
Other sources of bias	86%	9%	5%
Overall risk of bias	0%	30%	70%

The highest (bold italics), second highest (bold), and lowest (italics) proportion of studies categorized as low, unclear, or high risk of bias for each criterion is listed in the table along with the overall risk of bias.

### 3.4 | Organ system injury models and outcome reporting

Most studies (n = 53; 80%) assessed more than one outcome. A total of 63 studies (95%) reported benefit in at least one outcome and only 13 (20%) reported no benefit in at least one outcome. Only beneficial outcomes were reported in 53 studies (80%) while only no benefits were reported in only three studies (4.5%).<sup>20,41,42</sup> Only a single study reported an outcome with harm (Table 3).<sup>43</sup> Analysis of outcomes by organ system did not reveal any organ system for which ECFCs did not demonstrate benefit within this sample of studies. Refer to the table (Supporting Information 2) which outlines specific study characteristics and outcomes in more detail.

The most commonly modeled organ dysfunction was peripheral vascular ischemia (n = 29, 44%) and central nervous system injury (n = 14, 21%) (Table 3). Some organ injury categories included multiple types of organ dysfunction, while other categories were more homogenous in the induction of organ dysfunction. In particular, hind limb ischemia (n = 23),<sup>16-18,21,22,32,33,36,39,44-57</sup> cerebral ischemia (n = 7),<sup>12,37,58-62</sup> and acute renal injury (n = 6)<sup>11,23,26,27,30,63</sup> studies represent the three most common models of organ dysfunction (Table 4). Studies using hind limb ischemia as a model most often assessed revascularization by monitoring limb perfusion using ultrasound and reporting the ratio as a comparison with the unaffected limb.

Some studies also assessed histological and biochemical outcomes after sacrificing the animals. For cerebral ischemia, functional outcomes were most commonly assessed along with histological evidence of vascularization. Acute kidney injury models predominantly reported biochemical markers of kidney function and histology of renal tissue. The chief outcomes reported in these studies and whether benefit was observed is summarized in Table 4. For the



**FIGURE 3** Radar chart depicting the distribution of surface markers being reported across all articles. This chart is an aggregate of all markers reported within both the primary article and references protocols or previous studies

**TABLE 3** Organ systems injured in preclinical studies using ECFCs

Organ system modeled	n studies	Outcomes			
		Total	Benefit (%)	No benefit (%)	Harm (%)
Peripheral vascular	29	53	48 (91)	5 (9)	0 (0)
Central nervous system	14	42	39 (93)	3 (7)	0 (0)
Connective tissue	10	19	14 (74)	5 (26)	0 (0)
Cardiovascular	7	21	17 (81)	3 (14)	1 (5)
Renal	6	22	21 (95)	1 (5)	0 (0)
Musculoskeletal	4	6	6 (100)	0 (0)	0 (0)
Endocrine	5	12	11 (92)	1 (8)	0 (0)
Multiple system <sup>a</sup>	4	4	3 (75)	1 (25)	0 (0)
Respiratory	2	4	3 (75)	1 (25)	0 (0)
Immune	2	3	3 (100)	0 (0)	0 (0)
<b>Total</b>	<b>83<sup>b</sup></b>	<b>186</b>	<b>165 (89)</b>	<b>20 (11)</b>	<b>1 (1)</b>

<sup>a</sup>Multiple system refers to composite measures such as exercise capacity and changes in weight that cannot be isolated to an individual organ system.

<sup>b</sup>Some studies reported on outcomes related to one or more organ systems and the total number of studies is greater than 66.

Abbreviation: ECFCs, endothelial colony-forming cells.

**TABLE 4** Outcomes in three predominant models of organ dysfunction

<b>Hind limb ischemia</b> n = 23	Limb perfusion 22/1	Muscle injury score/necrosis 5/0	Capillary density 10/1	Other <sup>a</sup> 4/0
<b>Cerebral ischemia</b> n = 7	Neuro score/Maze tests 6/0	Somatosensory function 1/0	Neuron histology <sup>b</sup> 5 / 1	Capillary density 4/0
<b>Acute kidney injury</b> n = 6	Cr and/or BUN 6/0	Histology 6/0	Renal blood flow 0/1	Markers of inflammation 3/0

Note: Data are displayed as Number finding benefit/Number finding harm or no benefit.

<sup>a</sup>Apoptosis, limb function, gene expression;

<sup>b</sup>This includes blood brain barrier integrity.

Abbreviations: BUN, blood urea nitrogen; Cr, creatinine.

studies of hind limb ischemia, improved limb perfusion was reported in 22 of 23 studies with improved muscle injury scores in all five studies reporting this outcome. Capillary density was improved in 10 of 11 studies. Regarding studies of cerebral ischemia (n = 7), all six studies reporting on neurological scores and/or results of the maze test reported improvement with ECFCs, the only study reporting somatosensory function demonstrated improvement, and five of six studies reporting neuronal histology described beneficial changes. All six studies of acute kidney injury reported improved creatinine and blood urea nitrogen levels and favorable histological changes.

## 4 | DISCUSSION

Our systematic review of the literature provides useful insight into several aspects of controlled preclinical trials involving ECFCs. It highlights that research regarding the potential clinical application of this endothelial progenitor is still evolving rapidly, with most preclinical research emerging within the last decade or less. Additionally, our analysis confirms the relevance of ECFCs in ischemic models of tissue

injury and identifies robust cell characterization as an important aspect of future ECFC studies. Heterogeneity in reporting of ECFC characterization limits the ability to pool results from different studies; however, the use of a modified Medina criteria provides a basis for defining the minimal criteria needed for combining studies. While potential risk of bias was observed and publication bias was likely present due to the preclinical nature of studies, positive outcomes were reported in a large proportion of studies across a broad range of organ systems, providing encouragement that ECFC-based therapy holds promise in the treatment of vascular ischemia and other organ systems.<sup>2,3,64</sup> Future studies should embrace methods that reduce the potential for biased reporting, such as allocation concealment, randomization, and blinding of outcome assessors. Moreover, use of standardized criteria for ECFC characterization will allow greater confidence regarding efficacy of ECFC therapy in various injury models and will accelerate the translation of preclinical research to clinical trials of ECFC therapy.<sup>65</sup> ECFCs defined by the Medina 2017 consensus statement should be strongly considered as a unified standard as it relies on robust markers of surface expression which can be easily assessed by flow cytometry and a relatively simple functional assay of

tube forming capacity.<sup>5</sup> Additionally, beyond assessing intrinsic tube forming capacity, future studies should also consider characterizing ECFCs using a potency assays to further aid standardization and reproducibility.

Although our review clearly demonstrates the relevance of ECFCs in ischemic injury as a whole, we are unable to offer conclusions regarding which disease models should be prioritized for future trials. A significant proportion of studies used hind limb ischemia in their experimental model, this may reflect the utility of this model in assessing revascularization and functional recovery. Given the beneficial results in other models of ischemia injury, the applicability to clinical trials may not be restricted to peripheral vascular disease as much as acute ischemic injury more generally. Conversely, the surgical and/or mechanical induction of acute ischemic injury in the animal models may not reflect the clinical reality in most cases where the surrounding tissues and repair responses may also be implicated in any underlying disease process, particularly atherosclerotic ischemic injury that underpins most cerebral and cardiovascular ischemic events. Future preclinical trials should aim to explore the potential uses of ECFC in a greater variety of disease models that accurately reflect clinical scenarios to aid in the prioritization of indications for clinical trials.

The translation of preclinical research to clinical studies will also require greater attention to safety assessments which were largely lacking from the studies identified in our analysis. Cell-based therapies introduce the potential for off-target or unintended effects in other tissues and organs that need to be considered. Although the longitudinal intrinsic proliferative capacity of ECFCs *in vivo* has been assessed and has demonstrated relative safety in a few reports,<sup>66,67</sup> it remains reasonable to consider their ability to cause potential harm<sup>68</sup> related to persistence after repair is complete. The majority of preclinical studies in this review reported only the outcomes related to the injured organ system without specific consideration for other systems and the limited follow-up for outcome reporting in these studies precludes the ability to assess for potential long-term complications in the animals. Clarifying and anticipating these potential complications may further facilitate transition toward clinical trials.

Methodological issues related to the isolation, culture, and expansion of ECFCs are also potential barriers to translation that must be considered. Ideally, for early phase I/II clinical trials, ECFCs would be autologous, isolated from peripheral circulation, and expanded *ex vivo* in culture conditions that are compatible for human use. This would maximize safety to participants and minimize the presence of immunogenicity as a potential confounder. Protocols for successfully isolating and expanding ECFCs from unmodified whole blood using human platelet lysate in place of fetal bovine serum for culture supplementation have been described previously.<sup>69</sup> Furthermore, there is some evidence that ECFCs cultured with human platelet lysates may also exhibit greater vasculogenic capacity.<sup>70</sup> However, these protocols will likely require further optimization and adaptation to ensure the cells produced conform with Good Manufacturing Practices for cell therapies required for clinical trials within various jurisdictions.<sup>71</sup> Future studies should aim to expand their ECFCs in human compatible cultures whenever possible to mimic the eventual intervention to be

administered in clinical trials. Use of allogeneic cells such as those expanded from human umbilical cord blood may also be explored in human leukocyte antigen matched donor-recipient pairs and/or with immunosuppression.<sup>72</sup>

There are limitations of our review worth mentioning. Our initial literature search was broad to capture as many relevant articles as possible; however, it is possible that some published preclinical studies were not captured. Furthermore, our search and inclusion criteria were limited to articles published in English. It is clear even within our review that active research in this field is being conducted at an international level increasing the likelihood that there may be articles published in sources that were not captured in our search. It is also possible that we included in our analysis studies that did not truly use a cell phenotype that can be classified as an ECFC although we embraced criteria based on the consensus definition reported by Medina et al<sup>8</sup> and we included studies which reported on ECFC characterization either within the primary article or within referenced protocols or previous articles. By including studies that characterized ECFCs based on less stringent criteria than outlined by Medina 2017,<sup>8</sup> we introduced a degree of heterogeneity in cell characterization across the studies. As more studies embrace the full complement of criteria outlined by Medina et al, future analyses can focus only on studies that report the full set of established criteria.

## 5 | CONCLUSION

We conducted a systematic review which reveals that the body of evidence supporting the use of ECFCs as a potential cell-based regenerative therapy continues to grow rapidly with suggested benefit particularly in ischemic injuries across a range of organ systems including peripheral arterial occlusion, cerebral ischemia, and acute renal injury. However, the presence of potential reporting bias was observed and heterogeneity in cell-product characterization existed in the studies we identified in our analysis which precluded the ability to combine studies for meta-analysis. We suggest that future preclinical studies involving ECFCs be conducted with greater rigor to enable more rapid transition to clinical trials. This may be achieved by implementing standardized ECFC characterization based on the full Medina criteria which will reduce confusion in the literature, by incorporating methods to minimize sources of bias, by assessing for adverse outcomes in other tissue and at longer follow-up time points, and by exploring a greater variety of disease models that are clinically relevant.

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## CONFLICT OF INTEREST

D.S.A. declared consultant/advisory role for Canadian Blood Services. All the other authors declared no potential conflicts of interest.



## AUTHOR CONTRIBUTIONS

G.L., K.Z.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing and editing, approval of final manuscript; R.S.: administrative support, collection and assembly of data, approval of final manuscript; D.S.A.: conception and design, financial support, administrative support, manuscript writing and editing, approval of final manuscript.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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## SUPPORTING INFORMATION

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