Editorial

New Initiatives to Improve the Rigor and Reproducibility of Articles Published in *Circulation Research*

Roberto Bolli

The rampant irreproducibility of scientific articles is arguably the most serious problem that bedevils biomedical research.[1–12] It is a veritable plague that undermines the credibility of published studies, hinders clinical translation of basic work, and impedes the progress of medicine. Disquietingly, the problem seems to be getting worse rather than better.[10] Although the causes of irreproducibility are multifarious, inadequate rigor is probably the most important[2,11]; thus, it will be impossible to augment reproducibility without augmenting methodological rigor. It is not the purpose of this editorial to revisit the nature, origins, mechanisms, and consequences of irreproducibility, all of which have been discussed innumerable times in recent years, both in the literature and in ad hoc workshops.[1–12] There has been enough discussion; now it is time for action.

Much of this action must come from editors. Publication of methodologically flawed or suboptimal research can be limited by promulgating, and diligently applying, higher standards during editorial evaluation of submitted work. The editors of *Circulation Research* believe that the journal has a responsibility to implement initiatives that promote more rigorous and, therefore, more reproducible scientific work. It is for this reason that *Circulation Research* has published several reviews, editorials, Viewpoints, and News & Views articles focused on this issue.[1,2,7–12] It is for this reason that the journal has joined other leading journals to promote reproducibility of biomedical research[4] by endorsing the National Institutes of Health principles and guidelines for reporting preclinical research,[6] and it is for this very reason that we have recently chosen to publish a study showing that only a minority of preclinical animal experiments reported in leading cardiovascular journals (including *Circulation Research*) adheres to basic criteria necessary to assure rigor: for example, it was found that during a 10-year span, blinding was reported in 33% of the articles examined, randomization in 22%, and a priori sample size calculation in 2%.[7] The most troubling aspect of this report is that, with the exception of stroke research, there has been no improvement in the level of rigor in the past decade.[7]

The purpose of this editorial is to announce new initiatives aimed at enhancing the rigor, transparency, and reproducibility—and, thus, the overall robustness—of articles published in our journal. Three distinct initiatives will be implemented: checklists for rigor, measures to improve transparency, and a policy on refutations.

Checklists for Rigor

As of October 1, 2017, *Circulation Research* will require that authors fill out checklists to document the methodological rigor of preclinical work submitted for publication. Different checklists will be used for studies in animals and for studies in vitro because the challenges are different in the 2 settings. Although methodological weaknesses plague all domains of research, they seem to be particularly conspicuous in animal studies.[2,11,13] Accordingly, major emphasis in the checklists will be given to the methodology and reporting of investigations in animals.

Checklists for Studies in Animals

Authors will be asked to complete 2 checklists: a short one on initial submission and a longer one if and when the article is resubmitted in revised form. These documents are modifications of similar documents that were recently implemented by *Stroke*[3] and have resulted in improved adherence to standards of rigor.[7,14] The short checklist (Table 1) is relatively simple and covers the basic methodological aspects that are considered essential to the validity of the work: description of animals used, use of randomization, use of blinding, specification of inclusion and exclusion criteria and whether such criteria were established a priori, reporting of animals excluded, and description of statistical methods. The long checklist (Table 2) is much more detailed, providing information that enables in-depth evaluation of the level of rigor (and, therefore, robustness) of the study.

The short checklist will be used by reviewers and editors to evaluate the article but will not be published. The long checklist will be published as an online supplement along with the article; its purpose is to assist not only reviewers and editors, but also readers, to fully evaluate the extent to which the work adheres to standards of rigor. By examining the long checklist, readers will be able to assess for themselves the strengths and weaknesses of the study and how robust the data and conclusions are. The long checklist is accompanied by an explanatory document (Table 3), modeled after that used by *Stroke*,[3,14] which clarifies the standards of rigor outlined in the checklist and assists the authors in reporting them. We have elected to use 2 checklists because most articles submitted to *Circulation Research* are rejected at first submission; thus, requiring detailed information for every article that is submitted would be unnecessarily burdensome for the authors.

Checklist for Studies In Vitro

For molecular and cellular studies performed in vitro, authors will be required to fill out only one checklist if and when the

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Institute of Molecular Cardiology, University of Louisville, KY.
Correspondence to Roberto Bolli, MD, Institute of Molecular Cardiology, University of Louisville, ACB Third Floor, 550 S, Jackson St, Louisville, KY 40202. E-mail rbolli@louisville.edu

(*Circ Res*. 2017;121:472-479.
DOI: 10.1161/CIRCRESAHA.117.311678.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.117.311678

article is resubmitted in a revised form. This document (checklist for in vitro studies; Table 4) will be provided as a downloadable PDF file on the journal webpage and will cover the fundamental aspects that determine the rigor and robustness of the work, including technical replicates, characteristics of the antibodies and cell lines used, data processing and conversions, presentation of Western blots and immunofluorescence images, etc. This checklist will not be published, but authors will be asked to include the corresponding information in the text or in an online supplement. As in the case of the animal studies, the checklist will be accompanied by an explanatory document (Table 5), modeled after that used by *Stroke*,[3,14] which clarifies the standards of rigor outlined in the checklist and assists the authors in reporting them.

Although the new checklists clearly enunciate the desired standards of rigor, it is important to stress that failure to comply with some of the criteria outlined in these lists will not automatically or necessarily lead to rejection. Each study is different, and the weight that rigor has in the editorial decision must be individualized for each article. In the case of animal work, rigor is particularly important in validation studies—studies designed to verify the validity of a hypothesis in vivo (eg, whether a type of cell therapy, previously suggested to alleviate left ventricular dysfunction in exploratory studies, does indeed have such an effect). In contrast, some of the standards outlined in Tables 1 and 2 may not apply to exploratory, hypothesis-generating, or mechanistic animal studies (ie, studies where the primary goal is to explore the effect of an intervention in vivo for the first time, to obtain initial evidence in support of a hypothesis, or to elucidate a molecular or cellular mechanism). There are also gray zones. Many preclinical studies contain a combination of in vitro and in vivo data; the importance of the in vivo component varies greatly, and in some cases, these data may be peripheral to the main goal of the study. Thus, decisions on acceptance or rejection will continue to be made on a case-by-case basis depending on the specific content and features of each submitted article. If adherence to particular standards outlined in Tables 1, 2, and 4 was not appropriate or meaningful, we encourage the authors to address these issues in the text and to describe why this was the case.

Measures to Improve Transparency

In addition to the checklists, and in keeping with the goal of enhancing transparency,[6] we ask authors to use online supplements to provide detailed experimental protocols and communicate any secondary results not reported in the main article, both for in vivo and in vitro studies. As stated previously on these pages,[2,15] we expect all articles to describe the methods in sufficient detail to enable others to reproduce the work; this is usually achieved by publishing a detailed Methods section online. As recommended by the National Institutes of Health guidelines, authors should use online data supplements to describe "any similar experimental results that were omitted from the reporting for any reason, especially if the results do not support the main findings of the study" and "any outcomes or conditions that were measured or used and are not reported in the Results section."[6]

Policy on Refutations

Circulation Research also adheres to the National Institutes of Health recommendation that if an article is published in our journal, the journal "assumes responsibility to consider publication of refutations of that article, according to usual standards of quality."[6] In other words, an article that contradicts previous findings will not be penalized for not being the first; if the article is of quality consistent with the standards of *Circulation Research*, it will receive the same priority as if it were the first.

Other Considerations

The CAESAR Experience

An excellent example of the importance of methodological rigor can be found in the field of cardioprotection (limitation of myocardial infarct size), where thousands of therapies have been claimed to reduce infarct size during the past 5 decades, yet few have been reproducibly effective and (with the exception of early reperfusion) none has been translated into a clinical therapy.[11] The major reason for this colossal fiasco has been insufficient methodological rigor and transparency.[11] This problem was addressed in a workshop organized by the NHLBI in June 2003, which resulted in the implementation of a multicenter consortium (CAESAR) that conducted studies of putative cardioprotective therapies with a level of rigor comparable with that of randomized clinical trials; for example, with the use of randomization, blinding, a priori sample size and power calculation, etc.[16,17] When these methods were implemented, the CAESAR investigators found that different laboratories using the same protocols were able to obtain similar results.[16] It was also found that several therapies previously claimed to reduce infarct size were reproducibly ineffective in 3 different species, suggesting that an increased level of rigor eliminated false-positive results.[18,19]

Impact of the New Initiatives

The new checklists, transparency criteria, and refutations policy introduced herein are the most important changes in the editorial policies of *Circulation Research* in many decades. Their consequences will be profound and far reaching. It will take several years for their full impact to be realized, but this impact will be, I think, positive.

By promoting methodological rigor and transparency, these new guidelines will further enhance the already high quality of the work published in our journal, thereby augmenting its reproducibility and scientific utility. I expect that this will result in fewer controversies; therefore, less time, money, and effort will be consumed to resolve apparent conflicts in the literature. As fewer biased or inaccurate conclusions will be published, the number of positive studies will decrease dramatically. This, in turn, will enable investigators to focus on those ideas or hypotheses that are true (or likely to be true) rather than try to reproduce those that are not, which will speed up progress. Translation of preclinical results to clinical therapies will be facilitated because investigators will concentrate their efforts on those (few) interventions that are reproducibly effective in animal models and, thus, are more likely to be effective in patients. Importantly, there will be a moratorium, or possibly even a reversal, of the erosion of the credibility of research caused by irreproducible results.

Change is often difficult, and it seems likely that the guidelines enunciated in this article will meet with some opposition.

For example, it will be argued that they constitute yet another burden on authors in our overregulated world. Although I personally agree that our society is overregulated and that excessive paperwork and regulations are oppressive and can stifle creativity, I am more concerned about publishing conclusions that may be biased or inaccurate. The damage caused by the introduction of erroneous ideas in the literature greatly outweighs the extra effort necessary to comply with the principles that underlie the checklists.

An unintended consequence may be that these new checklists will discourage authors from submitting articles to *Circulation Research*. It is hoped that this will not happen, but it is a risk that must be taken. The alternative (publishing conclusions that may be inaccurate) is much worse. We have made a deliberate effort to minimize the burden on authors. For example, the initial checklist for animal studies (Table 1) is quite simple. Only when an article is seriously considered for publication is the long, more burdensome checklist (Table 2) required.

Time-Table for Implementation

To give authors time to reformat their papers in accordance with the new guidelines, the checklists will not be required until October 1, 2017.

What will happen then? After the launch of the initiatives outlined herein, we do not foresee a sudden spike in the level of rigor of the papers that we publish, but rather, a gradual increase. Manuscripts submitted to *Circulation Research* cannot be expected to conform with the new guidelines overnight. Obviously, studies that have already been completed cannot be modified, and changing the design and conduct of ongoing or planned experiments will take time. In many cases, 2-3 years elapse from the time when a study is first conceived to its submission for publication to our journal. Accordingly, in the short term we do not expect that, to be acceptable for publication, papers will have to meet all or almost all of the criteria specified in the checklists. We do hope, however, that these criteria will motivate investigators to change their practice in order to achieve a higher level of rigor, such that, in time, the rigor criteria encapsulated in these checklists (or at least most of them) will be met.

If this happens, it will be a slow process; it would be unrealistic to assume otherwise. The important thing, in our opinion, is to start this process. While our ultimate goal is to augment rigor and reproducibility in those papers that are published in *Circulation Research*, our more immediate objective is to promote a shift in experimental procedures toward practices that are conducive to a higher level of scientific validity. We will not be surprised if, over the next several months, we will continue to receive and publish manuscripts that do not meet all the criteria listed in the checklists. We will, however, be disappointed if, a few years from now, the level of rigor of studies published in *Circulation Research* has not improved.

Enhancing rigor, important though it may be, is not the only motivation for our actions. Even if a study does not meet the rigor criteria listed herein, the checklists will be helpful to the readers because they will allow them to see at a glance how much a study deviated from the ideal level of rigor and, therefore, how robust its conclusions are. An additional immediate benefit of the checklists is that they will spur authors to better report the details of their studies, which will enhance reproducibility irrespective of the level of rigor. Even if the experimental procedures do not change, a more complete, organized, and detailed description of the experiment will make it easier to either reproduce or refute it.

Concluding Remarks

The editors of *Circulation Research* are committed to upholding the highest standards of rigor and responsibility in both the conduct and the reporting of preclinical research. We hope that the new checklists and transparency criteria will clearly communicate the desired standards of rigor to authors and investigators while facilitating the journal's overall mission of promoting robust, rigorous, and reproducible scientific inquiry and experimentation.

Although adding more requirements for publication of articles in *Circulation Research* is not desirable, in the final analysis, the editors do not have a choice. Publication of studies that are less than rigorous (and, thus, inconclusive or possibly even misleading) does not benefit anyone, nor does dissemination of concepts that may be biased or inaccurate. As Ramirez et al[7] have pointed out, there has been no progress in rigor and transparency during the past decade, even after the promulgation of the National Institutes of Health guidelines[6] and the publication of many high-profile articles on the topic. The status quo is not acceptable. If we, as editors and gate keepers of scientific reporting, do not take action to remedy this problem, who will?

In conclusion, I think the initiatives outlined herein will enhance the quality of research published in *Circulation Research* and facilitate progress in cardiovascular biology. At present, most animal studies do not meet all of the criteria of rigor listed in Tables 1 and 2. In time, however, I think that the principles that underlie these guidelines will permeate the scientific community and become ingrained in the design of cardiovascular research, to the point that complying with the criteria articulated in the checklists will come natural. It will take time, but I think it will happen. When that happens, there will be less hype, less glamor, and probably less media headlines, but there will also be more truth. And that—the pursuit of the truth—is the core mission of any scientific journal.

Disclosures

None.

References

1. Bolli R. Dandum semper est tempus: the crucial importance of (and increasing disregard for) the test of time. *Circ Res*. 2015;117:755–757. doi: 10.1161/CIRCRESAHA.115.307613.
2. Bolli R. Reflections on the irreproducibility of scientific papers. *Circ Res*. 2015;117:665–666. doi: 10.1161/CIRCRESAHA.115.307496.
3. Vahidy F, Schäbitz WR, Fisher M, Aronowski J. Reporting standards for preclinical studies of stroke therapy. *Stroke*. 2016;47:2435–2438. doi: 10.1161/STROKEAHA.116.013643.
4. Journals unite for reproducibility. *Nature.* 2014;515–517. doi:10.1038/515007a.
5. Kilkenny C, Parsons N, Kadyszewski E, Festing MF, Cuthill IC, Fry D, Hutton J, Altman DG. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS One*. 2009;4:e7824. doi: 10.1371/journal.pone.0007824.
6. NIH. NIH Principles and Guidelines for Reporting Preclinical Research. https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research Accessed June 1, 2017.
7. Ramirez FD, Motazedian P, Jung RG, Di Santo P, MacDonald ZD, Moreland R, Simard T, Clancy AA, Russo JJ, Welch VA, Wells GA,

Hibbert B. Methodological rigor in preclinical cardiovascular studies: targets to enhance reproducibility and promote research translation. *Circ Res*. 2017;120:1916–1926. doi: 10.1161/CIRCRESAHA.117.310628.
8. Begley CG, Ioannidis JP. Reproducibility in science: improving the standard for basic and preclinical research. *Circ Res*. 2015;116:116–126. doi: 10.1161/CIRCRESAHA.114.303819.
9. Demer LL, Tintut Y, Nguyen KL, Hsiai T, Lee JT. Rigor and reproducibility in analysis of vascular calcification. *Circ Res*. 2017;120:1240–1242. doi: 10.1161/CIRCRESAHA.116.310326.
10. Williams R. Can't get no reproduction: leading researchers discuss the problem of irreproducible results. *Circ Res*. 2015;117:667–670. doi: 10.1161/CIRCRESAHA.115.307532.
11. Bolli R, Becker L, Gross G, Mentzer R Jr, Balshaw D, Lathrop DA; NHLBI Working Group on the Translation of Therapies for Protecting the Heart from Ischemia. Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res*. 2004;95:125–134. doi: 10.1161/01.RES.0000137171.97172.d7.
12. Jones SP. I'll have the rigor, but hold the mortis. *Circ Res*. 2017;120:1852–1854. doi: 10.1161/CIRCRESAHA.117.311114.
13. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the arrive guidelines for reporting animal research. *PLoS Biol*. 2010;8:e1000412.
14. Minnerup J, Zentsch V, Schmidt A, Fisher M, Schäbitz WR. Methodological quality of experimental stroke studies published in the stroke journal: time trends and effect of the basic science checklist. *Stroke*. 2016;47:267–272. doi: 10.1161/STROKEAHA.115.011695.
15. Bolli R. The new circulation research: a manifesto. *Circ Res*. 2010;106:216–226. doi: 10.1161/RES.0b013e3181d39f39.
16. Jones SP, Tang XL, Guo Y, et al. The NHLBI-sponsored consortium for preclinical assessment of cardioprotective therapies (CAESAR): a new paradigm for rigorous, accurate, and reproducible evaluation of putative infarct-sparing interventions in mice, rabbits, and pigs. *Circ Res*. 2015;116:572–586. doi: 10.1161/CIRCRESAHA.116.305462.
17. Lefer DJ, Bolli R. Development of an NIH consortium for preclinical assessment of cardioprotective therapies (CAESAR): a paradigm shift in studies of infarct size limitation. *J Cardiovasc Pharmacol Ther*. 2011;16:332–339. doi: 10.1177/1074248411414155.
18. Kukreja R, Tang XL, Lefer D, et al. Administration of sildenafil at reperfusion fails to reduce infarct size: results from the CAESAR cardioprotection consortium (LB650). *The FASEB Journal*. 2014;28(1 suppl). http://www.fasebj.org/content/28/1_Supplement/LB650.short.
19. Lefer D, Jones S, Steenbergen C, et al. Sodium nitrite fails to limit myocardial infarct size: results from the CAESAR cardioprotection consortium (LB645). *The FASEB Journal*. 2014;28(1 suppl). http://www.fasebj.org/content/28/1_Supplement/LB645.short.

KEY WORDS: Editorials ■ biomedical research ■ checklist ■ sample size ■ stroke

Table 1. Short (Initial) Checklist of Methodological and Reporting Aspects for Articles Submitted to *Circulation Research* That Report Studies of Experimental Interventions in Animals

Preclinical testing	Prevention of bias is important for experimental cardiovascular research. For studies where the primary objective is the preclinical testing of therapies, the following checklist items must be adhered to and clearly presented in the article. Study involves testing of therapeutic or diagnostic agent in animal models: Yes/No
Animals	Species, age, sex, strains, and sources of animals are described: yes/no
Randomization	Randomization and allocation concealment were performed: yes/no
Blinding	Blinding was performed: yes/no
Inclusions and exclusions	Specific criteria for inclusions and exclusions are specified: yes/no
	Criteria for inclusions and exclusions were set before the study: yes/no
Reporting of excluded animals	All animals excluded after randomization are reported: yes/no
Statistical methods	Statistical methods are described: yes/no

Table 2. Checklist of Methodological and Reporting Aspects for Articles Submitted to *Circulation Research* That Report Studies of Experimental Interventions in Animals

Methodological and Reporting Aspects	Description of Procedures
Study design	□ The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study.
	□ An overall study timeline is provided.
	□ The protocol was prospectively written.
	□ The primary and secondary end points are specified.
	□ For primary end points, a description is provided as to how the type I error multiplicity issue was addressed (eg, correction for multiple comparisons was or was not used and why). (Note: correction for multiple comparisons is not necessary if the study was exploratory or hypothesis generating in nature).
	□ A description of the control group is provided including whether it matched the treated groups.
Inclusion and exclusion criteria	□ Inclusion and exclusion criteria for enrollment into the study were defined and are reported in the article.
	□ These criteria were set a priori (before commencing the study).
Randomization	□ Animals were randomly assigned to the experimental groups. If random assignment was not used, adequate explanation has been provided.
	□ Type and methods of randomization have been described.
	□ Allocation concealment was used.
	□ Methods used for allocation concealment have been reported.

(*Continued*)

Table 2. Continued

Methodological and Reporting Aspects	Description of Procedures
Blinding	☐ Blinding procedures with regard to masking of group/treatment assignment from the experimenter were used and are described. The rationale for nonblinding of the experimenter has been provided, if such was not performed.
	☐ Blinding procedures with regard to masking of group assignment during outcome assessment were used and are described.
	☐ If blinding was not performed, the rationale for nonblinding of the people analyzing outcome has been provided.
Sample size and power calculations	☐ Formal sample size and power calculations were conducted before commencing the study based on a priori determined outcome(s) and treatment effect(s), and the data are reported.
	☐ If formal sample size and power calculation was not conducted, a rationale has been provided.
Data reporting	☐ Baseline characteristics (species, sex, age, strain, chow, bedding, and source) of animals are reported.
	☐ The number of animals in each group that were randomized, tested, and excluded and died is reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided for all experimental groups.
	☐ Baseline data on assessed outcome(s) for all experimental groups are reported.
	☐ Details on important adverse events and death of animals during the course of the experiment are reported for all experimental groups.
	☐ Numeric data on outcomes are provided in the text or in a tabular format in the main article or as supplementary tables, in addition to the figures.
	☐ To the extent possible, data are reported as dot plots as opposed to bar graphs, especially for small sample size groups.
	☐ In the online supplemental material, methods are described in sufficient detail to enable full replication of the study.
Statistical methods	☐ The statistical methods used for each data set are described.
	☐ For each statistical test, the effect size with its standard error and *P* value is presented. Authors are encouraged to provide 95% confidence intervals for important comparisons.
	☐ Central tendency and dispersion of the data are examined, particularly for small data sets.
	☐ Nonparametric tests are used for data that are not normally distributed.
	☐ 2-sided *P* values are used.
	☐ In studies that are not exploratory or hypothesis generating in nature, corrections for multiple hypotheses testing and multiple comparisons are performed.
	☐ In negative studies or null findings, the probability of a type II error is reported.
Experimental details, ethics, and funding statements	☐ Details on experimentation, including formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring, are described.
	☐ Both male and female animals have been used. If not, the reason/justification is provided.
	☐ Statements on approval by ethics boards and ethical conduct of studies are provided.
	☐ Statements on funding and conflicts of interests are provided.

The above checklist is a modified version of a similar checklist promulgated by *Stroke* in 2011 and revised and expanded in 2016. The purpose of the checklist is to provide important metrics for the conduct and reporting of preclinical studies that involve assessment of experimental interventions in vivo. The criteria listed herewith are intended to (i) clearly communicate the desired standards of rigor to authors and investigators and (ii) facilitate the journal's overall mission of promoting robust, rigorous, and reproducible scientific inquiry and experimentation. We recognize the complexity and diversity of many studies, and the fact that different studies have different levels of emphasis on in vivo data. The information requested herein is meant to be used by reviewers and editors as an aid in assessing the value of the work, not as a rigid criterion for accepting or rejecting articles; thus, decisions on acceptance or rejection will be made on a case-by-case basis depending on the individual content and characteristics of each submitted article. In addition, we recognize that certain standards outlined above may not apply to exploratory, hypothesis-generating, or mechanistic animal studies. We encourage and welcome such studies; however, in these cases we encourage the authors to address the areas outlined here and describe why adherence to particular standards was not practical or meaningful. We promote the use of online supplements for providing detailed experimental protocols and communicating any secondary results not reported in the main article.

Table 3. Explanatory Accompaniment to the Checklist for Methodological and Reporting Features of Studies Involving Experimental Interventions in Animals

Study design	The protocol should be written before commencing the study. If the study is exploratory (eg, the first test of a new therapy or manipulation in vivo), or if it is primarily mechanistic (ie, its primary purpose is to elucidate mechanisms rather than establish efficacy), definition of primary and secondary end points is not necessary. However, if the study is confirmatory (ie, its purpose is to validate the effect of a therapy or intervention), primary and secondary end points should be defined and reported. Furthermore, if the study is confirmatory and if multiple groups are compared with respect to the primary end point, corrections for a type I error should be used.
Inclusion and exclusion criteria	A priori defined criteria to include and exclude animals need to be established and described. These may be various parameters like animals' species, sex, strain, weight, developmental state, source nomenclature, and genotype, drug or test naive, and so on. Any criteria used for inclusion/exclusion after the protocol is started (eg, loss of cardiac function after infarction) should also be reported.

(*Continued*)

Table 3. Continued

Randomization	Randomization is considered the gold standard for studies intended to test hypotheses and should be used for assignment of animals to control or experimental groups. Certain studies, such as those with only a single arm (intervention only) or those with exploratory or mechanistic aims (vide supra), may not need to use randomization. Authors are asked to provide clear explanations of such exceptions. The type and methods used to randomize animals to various study arms should be reported. a. Type of randomization: for example, simple or stratified. If stratified, please describe which variables/factors were used for randomization and why. It is also recommended that investigators report measures of successful randomization, by providing baseline characteristics of animals in various comparison groups. b. Process of randomization: please provide details of the process used for randomization. These may include (but are not limited to) use of electronically generated lists, sealed envelopes, coin toss, dice roll, etc. c. Other procedural details pertaining to randomization include describing the independence of team members that generate randomization sequences, perform randomization and group allocation, and conduct experimentation. Allocation concealment is an important aspect of randomization. If the study team member(s) performing randomization have knowledge of treatment assignment groups before selection of animals, then the allocation is not concealed, and a selection bias can be introduced. Allocation concealment can be achieved if deidentified codes are generated and provided to team members performing the randomization. This usually entails an independent team member producing randomization sequences and lists. The journal encourages use and reporting of such procedures.
Blinding	Blinding is essential to avoid bias and must be used whenever possible. Blinding or masking refers to a. Inability of the investigator(s) that administers the study intervention to discern treatment from the placebo/vehicle. This can be achieved by providing the investigator(s) with animals having codes for group allocation, as well as study drug/product and placebo using a concealment procedure. The journal strongly recommends using and reporting the blinding procedures used for administration of treatment/placebo, wherever possible. For experimentation that in itself is surgical in nature, masking of the investigator(s) performing the study intervention may not be possible. We encourage appropriate reporting of these aspects in the article. b. Besides the masking of intervention, it is also important that all outcomes be assessed in a blinded manner. Although blinding the investigator(s) who administers the treatment may not be possible in all instances, blinded assessment of functional (eg, echocardiographic), histological (eg, infarct size), and other outcomes is almost universally possible. This is achieved by using an effective tracking mechanism for the experimental animals and via independence of the study team members performing outcome ascertainment from those administering the study intervention. Authors are expected to provide an account of the experimental phases that were blinded to various investigator(s) and the steps undertaken to maintain blinding while transitioning from one experimental phase to the other.
Sample size and power calculations	In confirmatory studies involving formal hypothesis testing (vide supra), authors should provide an explanation of power and sample size calculations. The rationale for using the chosen sample size should be provided. The rationale for postulating a certain effect size of the intervention on an a priori-determined primary outcome(s) should be presented, along with the anticipated variability. The sources that form the bases of power and sample size calculations should be cited. We do recognize that in exploratory or mechanistic experiments (vide supra), a formal power and sample size calculation may not be possible or meaningful. These exploratory or mechanistic analyses should be presented as such, and an explanation of why power calculations were not formally performed should be provided.
Data reporting	Control groups may either be placebo concurrent, no-treatment concurrent, active-treatment concurrent, or dose/timing-comparison concurrent. This needs to be clearly stated. It is required that investigators use a concurrent control for each intervention group. Using singular control data across several experiments conducted during a course of time is similar to using historical controls in clinical research and is a potential source of error. The number of animals included and analyzed in intervention and control groups should be clearly reported. The authors may consider using a flow diagram (as supplemental material) showing the number of animals available for randomization, number of animals randomized to various treatment/control groups, number of animals in which baseline data were collected, number of animals included in the intervention, number of animals in which follow-up/outcomes were assessed, and number of animals that were lost at any stage during the experimental protocol. Animal characteristics, such as age, developmental stage, sex, species, genetic strain, and comorbidities, should be reported, along with relevant baseline data (eg, baseline left ventricular function). Number of deaths or other events leading to exclusion should be reported in all experimental groups. If none, please state so. When reporting results, investigators are encouraged to provide summary data as tables (ie, authors should not limit data to figures). At times, figures in themselves do not allow for precise reporting of measures of central tendency and spread for different time points. If data are presented as figures, tabulated results can be provided in supplementary materials. When sample sizes are small, dot plots are preferable to bar graphs because they provide more information on variability.
Statistical methods	Authors are required to provide details on the statistical methods used for each analysis and hypothesis tested. Descriptive analyses should use measures of central tendency and spread suitable to the distribution of the data. Measures of spread (eg, SD or SE) should be suitably used, reported, and interpreted. Use of 95% confidence intervals is important to assess the size of the effect of interventions. Nonparametric tests are more appropriate for data that are not distributed normally. Commonly used tests, such as the Student t test, might not be appropriate when the sample size in each group is small. The levels of significance used for hypothesis testing should be reported, and if any adjustments were done for multiple or repeated testing. Corrections for multiple hypotheses testing and multiple comparisons to avoid type I errors are essential, unless the study is exploratory or mechanistic in nature (vide supra). When the primary outcome of a study is negative, the probability of a type II error should be reported because sample sizes are often insufficient to rule out the null hypothesis with a high level of confidence.

(*Continued*)

Table 3. Continued

Experimental details, ethics, and funding statements	It is important to provide as many experimental details as necessary for interpretation, comparison, and, importantly, full replication of the study. These details include (but are not limited to) formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring. Temperature is particularly important in open-chest models. Further details on primary and secondary outcomes and methods used for analysis of outcomes should also be provided or clearly referenced. Authors should give due consideration to the use of animals with both sexes. Assuming only males or females are used, reasons need to be provided. The journal requires that all studies reporting animal experimentation abide by the respective institutional animal welfare and ethics regulations and reporting of approvals. Funding and conflict of interest statements should also be provided. The funding of the study by a drug development or technology development industry should be clearly stated. Any financial interest of the authors in the product being tested should be clearly indicated.

The above table is a modified version of a similar table promulgated by *Stroke* in 2011 and revised and expanded in 2016. The purpose of the table is to provide important metrics for the conduct and reporting of preclinical studies that involve assessment of experimental interventions in vivo. The recommendations provided herewith are intended to (i) clearly communicate the desired standards of rigor to authors and investigators and (ii) facilitate the journal's overall mission of promoting robust, rigorous, and reproducible scientific inquiry and experimentation. We recognize the complexity and diversity of many studies, and the existence of different levels of emphasis on in vivo data in different studies. The recommendations outlined herein are meant to be used by reviewers and editors as guidelines, not as rigid criteria for accepting or rejecting articles; thus, decisions on acceptance or rejection will continue to be made on a case-by-case basis depending on the individual content and message of each submitted article. In addition, we recognize that certain standards outlined above may not apply to exploratory, hypothesis-generating, or mechanistic animal studies. We encourage and welcome such studies; however, in these cases, we encourage the authors to address the areas outlined here and describe why adherence to particular standards was not practical or meaningful. We promote the use of online supplements for providing detailed experimental protocols and communicating any secondary results not reported in the main article.

Table 4. Checklist of Methodological and Reporting Aspects for Articles Submitted to *Circulation Research* That Report Molecular and/or Cellular Studies In Vitro

Methodological and Reporting Aspects	Description of Procedures
Conduct of the study	□ Technical replicates, whenever applicable, such as RNA-sequencing and ChIP experiments, are described.
	□ Sufficient information about sample collection is provided to distinguish between independent biological data points and technical replicates.
	□ Specific characteristics of each antibody used, such as source, host, immunoglobulin fragment, titer, catalog number, and how the antibody was validated, are described.
	□ Specific characteristics of each cell line used, such as source, authentication, and passage number, are described.
	□ Any data processing and conversions are described.
Data reporting	□ In Western blots, all experimental and control groups are included in the same blot.
	□ Molecular size markers are included in all blots.
	□ Immunofluorescence imaging is processed similarly in the experimental and control groups.
	□ A control secondary antibody or IgG is included in immunofluorescence imaging.
	□ Scale bars have been added to all micrographs.
	□ All units of measurements are specified.
	□ To the extent possible, data are reported as dot plots as opposed to bar graphs, especially for small sample size groups.
	□ In the online supplemental material, methods are described in sufficient detail to enable full replication of the study.
Statistical methods	□ The statistical methods used for each data set are described.
	□ For each statistical test, the effect size with its standard error, 95% confidence interval (for important comparisons), and *P* value is presented.
	□ Central tendency and dispersion of the data are examined, particularly for small data sets.
	□ Nonparametric tests are used for data that are not normally distributed.
	□ 2-sided *P* values are used.
	□ In studies that are not exploratory in nature, corrections for multiple hypotheses testing and multiple comparisons are performed.

The purpose of this checklist is to provide important metrics for the conduct and reporting of molecular and cellular studies in vitro. The criteria listed herewith are intended to (i) clearly communicate the desired standards of rigor to authors and investigators and (ii) facilitate the journal's overall mission of promoting robust, rigorous, and reproducible scientific inquiry and experimentation. The information requested herein is meant to be used by reviewers and editors as an aid in assessing the value of the work, not as a rigid criterion for accepting or rejecting articles; thus, decisions on acceptance or rejection will be made on a case-by-case basis depending on the individual content and message of each submitted article. In addition, we recognize that certain standards outlined above may not apply to exploratory, hypothesis-generating, or mechanistic studies. In these cases, we encourage the authors to address the criteria outlined here and describe why adherence to particular standards was not practical or meaningful. We ask authors to use online supplements for providing detailed experimental protocols and communicating any secondary results not reported in the main article.

Table 5. Explanatory Accompaniment to the Checklist for Methodological and Reporting Features of Molecular and/or Cellular Studies In Vitro

Data reporting	When reporting results, investigators are encouraged to provide summary data as tables, whenever applicable (ie, authors should not limit data to bar graphs). At times, the data presented as bar graphs in themselves do not allow for precise reporting of measures of central tendency and spread for different time points. If data are presented as bar graphs, tabulated results can be provided in supplementary materials. When sample sizes are small, dot plots are preferable to bar graphs because they provide more information on variability. Please indicate that large datasets, such as RNA-Seq and other omics, will be deposited to the appropriate public databases.
Statistical methods	Authors are required to provide details on the statistical methods used for each analysis and hypothesis tested. Descriptive analyses should use measures of central tendency and spread suitable to the distribution of the data. Measures of spread (eg, SD or SE) should be suitably used, reported, and interpreted. The 95% confidence intervals are important to assess the size of the effect of interventions and should be specified for comparisons that are deemed important. Nonparametric tests are more appropriate for data that are not distributed normally. Commonly used tests, such as the Student t test, might not be appropriate when the sample size in each group is small. The levels of significance used for hypothesis testing, and if any adjustments were done for multiple or repeated testing, should be reported. Corrections for multiple hypotheses testing and multiple comparisons to avoid type I errors are essential, unless the study is exploratory or mechanistic in nature (vide supra). When the primary outcome of a study is negative, the probability of a type II error should be reported because sample sizes are often insufficient to rule out the null hypothesis with a high level of confidence.
Experimental details, ethics, and funding statements	It is important to provide as many experimental details as necessary for interpretation, comparison, and, importantly, full replication of the study. Any financial interest of the authors in the product being tested should be clearly indicated.

The above table is a modified version of a similar table promulgated by *Stroke* in 2011 and revised and expanded in 2016. The purpose of the table is to provide important metrics for the conduct and reporting of molecular and/or cellular studies in vitro. The recommendations provided herewith are intended to (i) clearly communicate the desired standards of rigor to authors and investigators and (ii) facilitate the journal's overall mission of promoting robust, rigorous, and reproducible scientific inquiry and experimentation. The recommendations outlined herein are meant to be used by reviewers and editors as guidelines, not as rigid criteria for accepting or rejecting articles; thus, decisions on acceptance or rejection will continue to be made on a case-by-case basis depending on the individual content and characteristics of each submitted article. We promote the use of online supplements for providing detailed experimental protocols and communicating any secondary results not reported in the main article.