

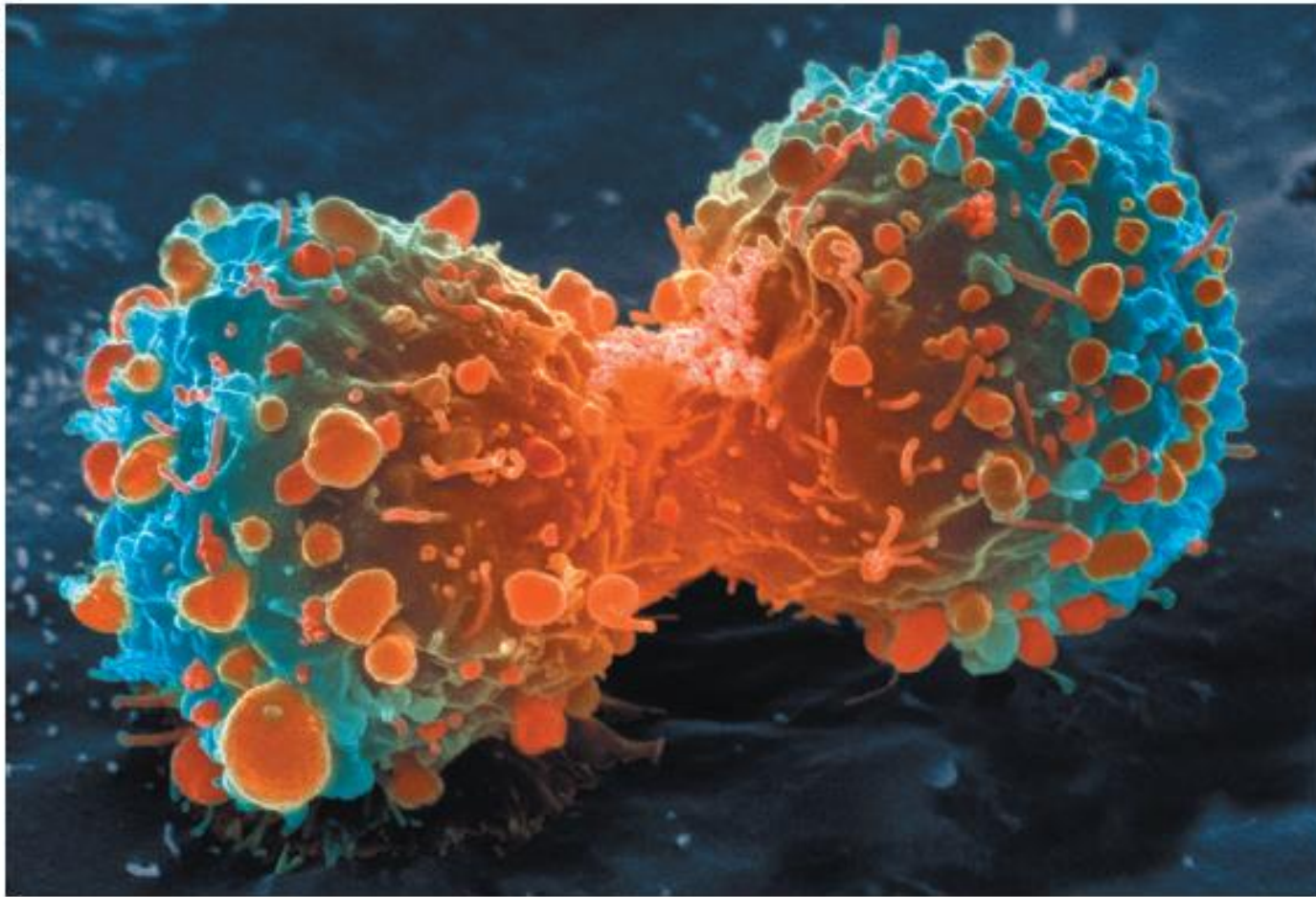
Analyzing the Scientific Literature: Pitfalls and Reproducibility

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Many landmark findings in preclinical oncology research are not reproducible, in part because of inadequate cell lines and animal models.

Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

Begley and Ellis - Amgen Analysis

- ▶ Chose 53 key publications related to oncology
- ▶ Could only reproduce the findings of 6 publications
- ▶ Rarely due to intentional fraudulent behavior
- ▶ Individual sloppiness, scientific laziness, ignorance
exaggeration, desperation

REPRODUCIBILITY OF RESEARCH FINDINGS

Preclinical research generates many secondary publications, even when results cannot be reproduced.

Journal impact factor	Number of articles	Mean number of citations of non-reproduced articles*	Mean number of citations of reproduced articles
>20	21	248 (range 3-800)	231 (range 82-519)
5-19	32	169 (range 6-1,909)	13 (range 3-24)

Results from ten-year retrospective analysis of experiments performed prospectively. The term 'non-reproduced' was assigned on the basis of findings not being sufficiently robust to drive a drug-development programme.

*Source of citations: Google Scholar, May 2011.

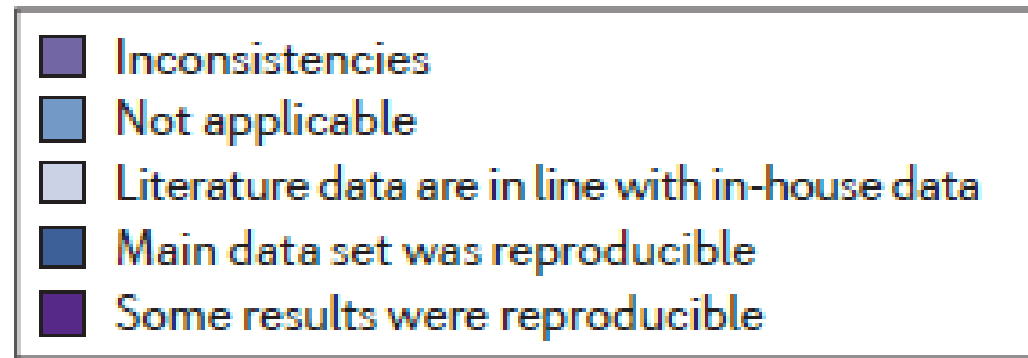
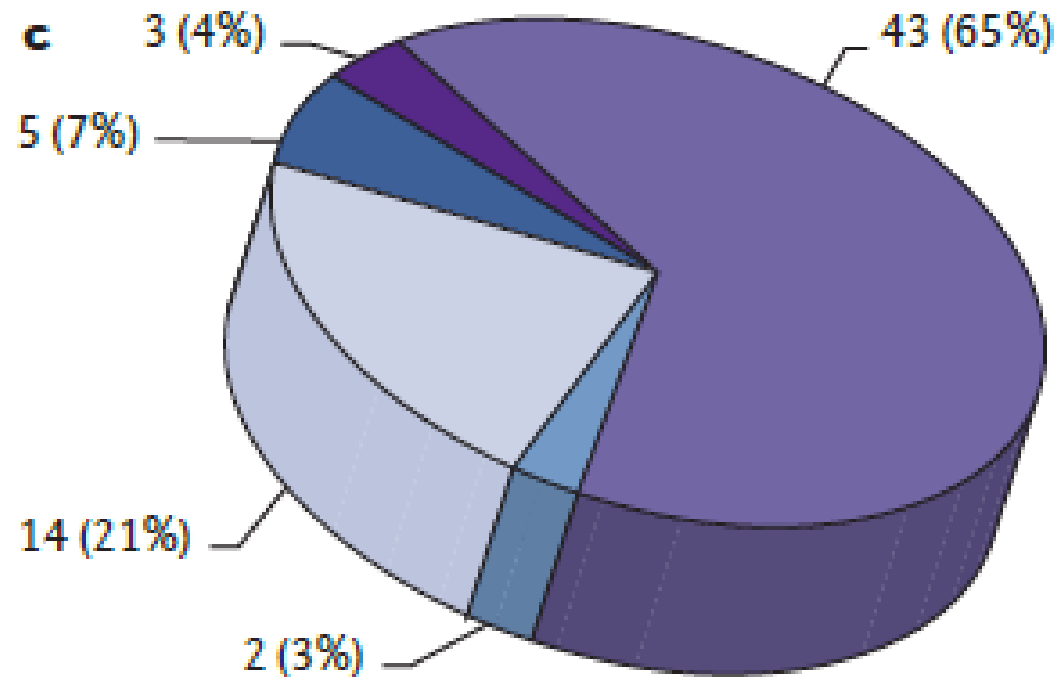
**The first principle is that you
must not fool yourself - and
you are the easiest
person to fool.**

▶ Richard Feynman

Believe it or not: how much can we rely on published data on potential drug targets?

Florian Prinz, Thomas Schlange and Khusru Asadullah

At Least 65% Not Reproducible



IS THERE A REPRODUCIBILITY CRISIS?

A *Nature* survey lifts the lid on how researchers view the 'crisis' rocking science and what they think will help.

BY MONYA BAKER

52%
Yes, a significant
crisis

3%
No, there is no crisis

7%
Don't know

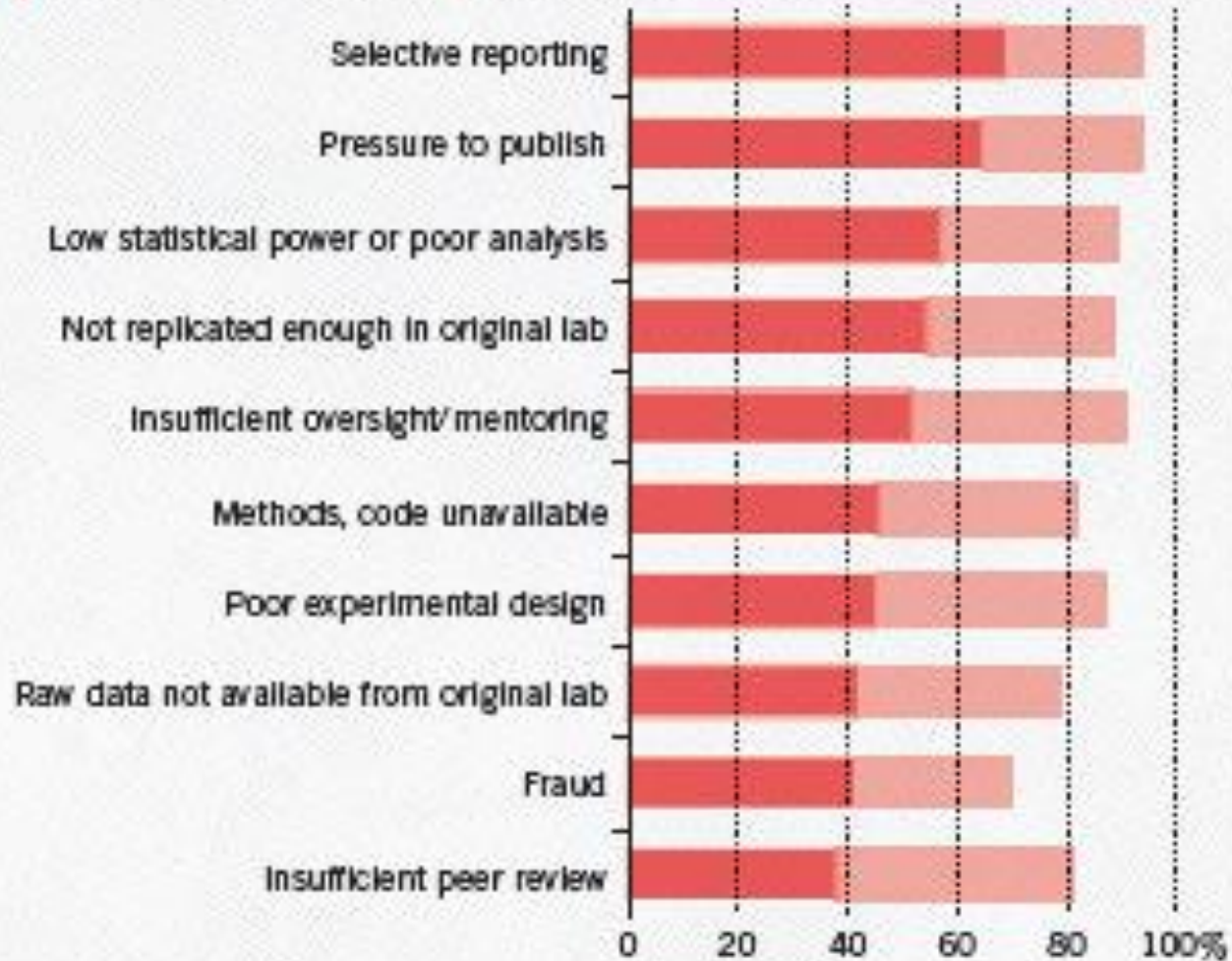
38%
Yes, a slight
crisis

1,576
RESEARCHERS SURVEYED

WHAT FACTORS CONTRIBUTE TO IRREPRODUCIBLE RESEARCH?

Many top-rated factors relate to intense competition and time pressure.

● Always/often contribute ● Sometimes contribute



Perspective

The Increasing Urgency for Standards in Basic Biologic Research

Leonard P. Freedman¹ and James Inglese²

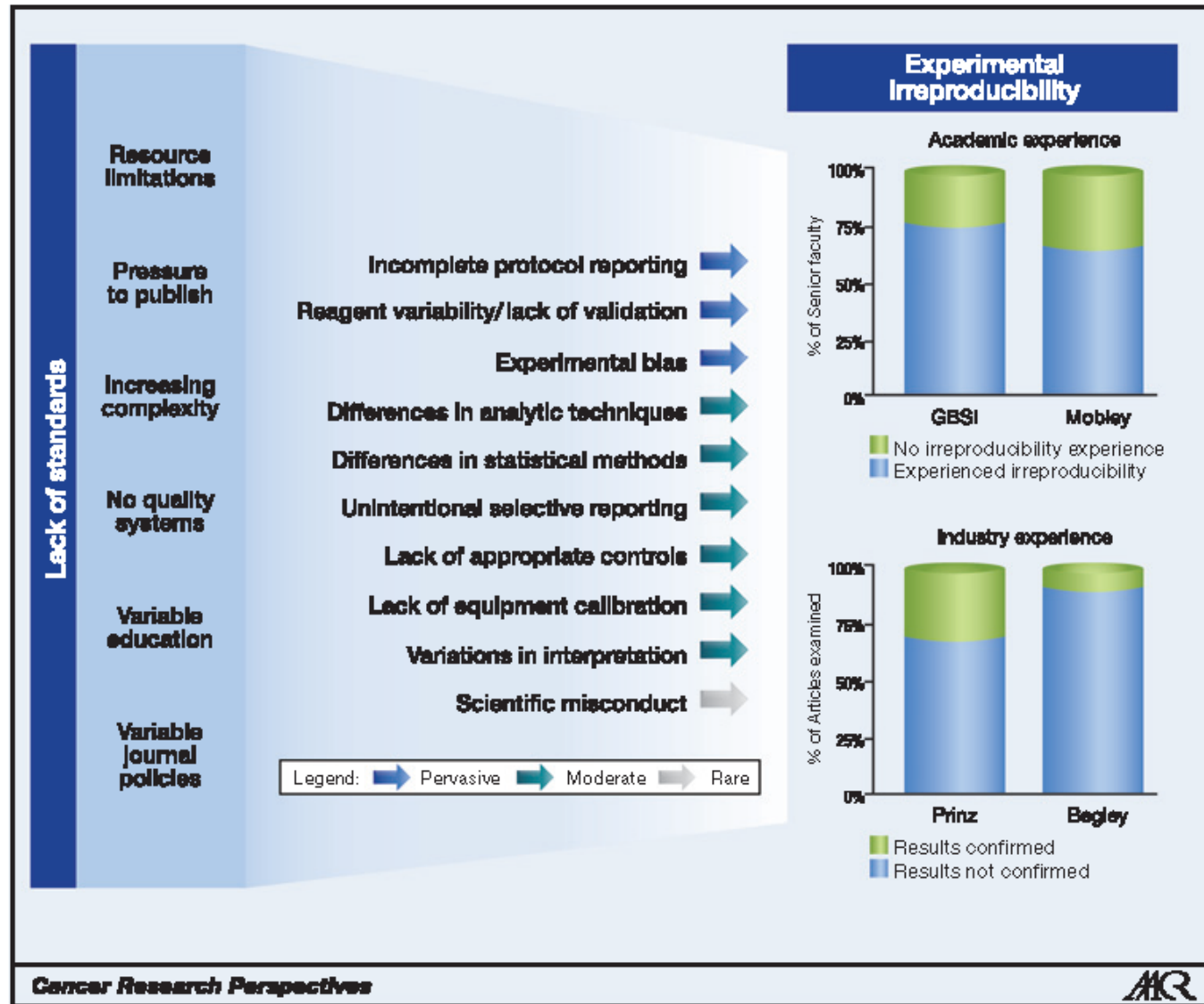


Figure 1. Causes of irreproducibility. Academic experience refers to personal experience of academic senior faculty with irreproducibility (3) and interviewee responses (8). Industry experience refers to the percentage of articles studies reported being able to reproduce (1, 2). Causes of irreproducibility defined and ranked on the basis of GBSI interviews (8), Loscalzo (38) and Begley and Ellis (1); source, GBSI (8).

Why Most Published Research Findings Are False

John P. A. Ioannidis

- ▶ Statistical Errors
 - ▶ Inadequate statistical power
 - ▶ Inappropriate statistical analysis

Another Cause of False Published Findings: Incorrect Pathologic Diagnoses

Peroxisome proliferator-activated receptor γ agonist troglitazone induces colon tumors in normal C57BL/6J mice and enhances colonic carcinogenesis in *Apc*^{1638 N/+} *Mlh1*^{+/-} double mutant mice

Kan Yang¹, Kun-Hua Fan¹, Sergio A. Lamprecht¹, Winfried Edelmann², Levy Kopelovich³, Raju Kucherlapati⁴ and Martin Lipkin^{1*}

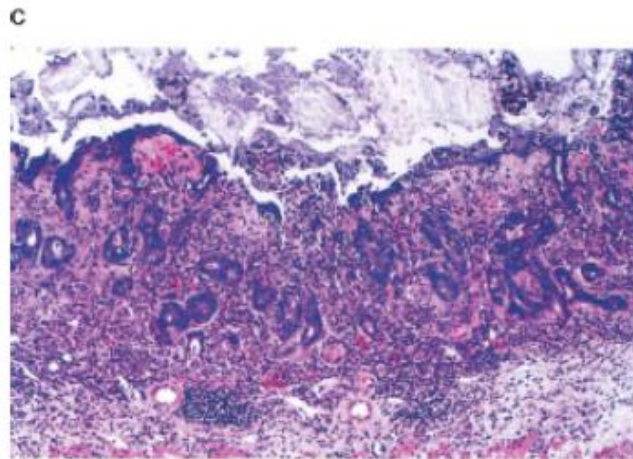
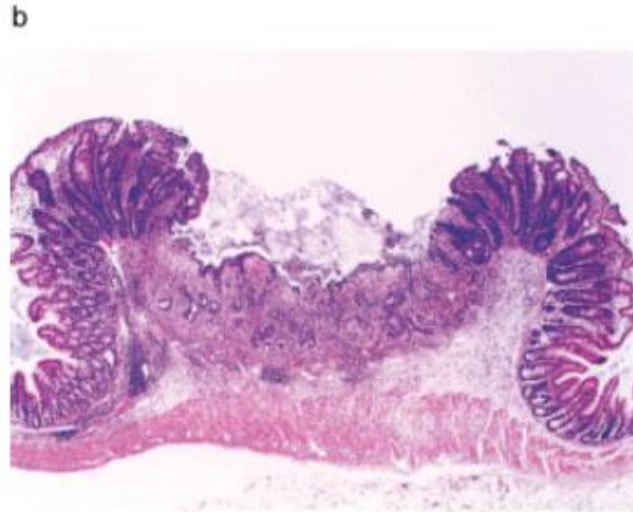
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²*Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA*

³*Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA*

⁴*Division of Genetics, Brigham and Women Hospital, Boston, MA, USA*

Benign Colonic Ulcer with Regeneration: Diagnosed as Invasive Carcinoma.



Basic causes of Irreproducibility

- ▶ Inadequate protocol definition
- ▶ Inappropriate model
- ▶ Statistical power and analysis
- ▶ Inappropriate pathology review
- ▶ Inappropriate animal model
- ▶ Cell line misidentification
- ▶ Definition of reagents (e.g. antibodies)
- ▶ Quality control of experimental materials
- ▶ Lack of randomization
- ▶ Inappropriate controls
- ▶ Confirmation bias
- ▶ **Major Bias: Lack of Blinding in Evaluation of Assays**

Issues with Animals

- ▶ Specific pathogen free (SPF)
- ▶ Age, strain, sex, species
- ▶ Randomization

Often Overlooked Variables in Animal Experiments

- ▶ Time of day - diurnal variation
- ▶ Fasting vs. non-fasting
- ▶ Randomization
- ▶ Sequential sampling between groups
- ▶ Proper controls - negative and positive

Cell Lines

- ▶ Cell line identification (contamination)
- ▶ Passage number
- ▶ Mycoplasma, other contaminants
- ▶ Appropriate cell type
- ▶ Primary vs. immortalized
- ▶ Metabolic capability
- ▶ Presence of appropriate receptors

Is the Animal or Cell Model Relevant to Humans?

Models: All are wrong.

Some are useful.

~George Box

Key Issues in Epidemiology

- ▶ Prospective vs. Retrospective
- ▶ Sample size (statistical power)
- ▶ Exposure analysis
- ▶ Confounding factors (nutrition, smoking, other exposures, etc.)
- ▶ Methods for comparison (continuous variable, x-tiles, highest vs. lowest)
- ▶ Publication bias (Kavvoura et al., PLoS Med., 4: e79, 2007)

Stresses Against Evaluating Reproducibility

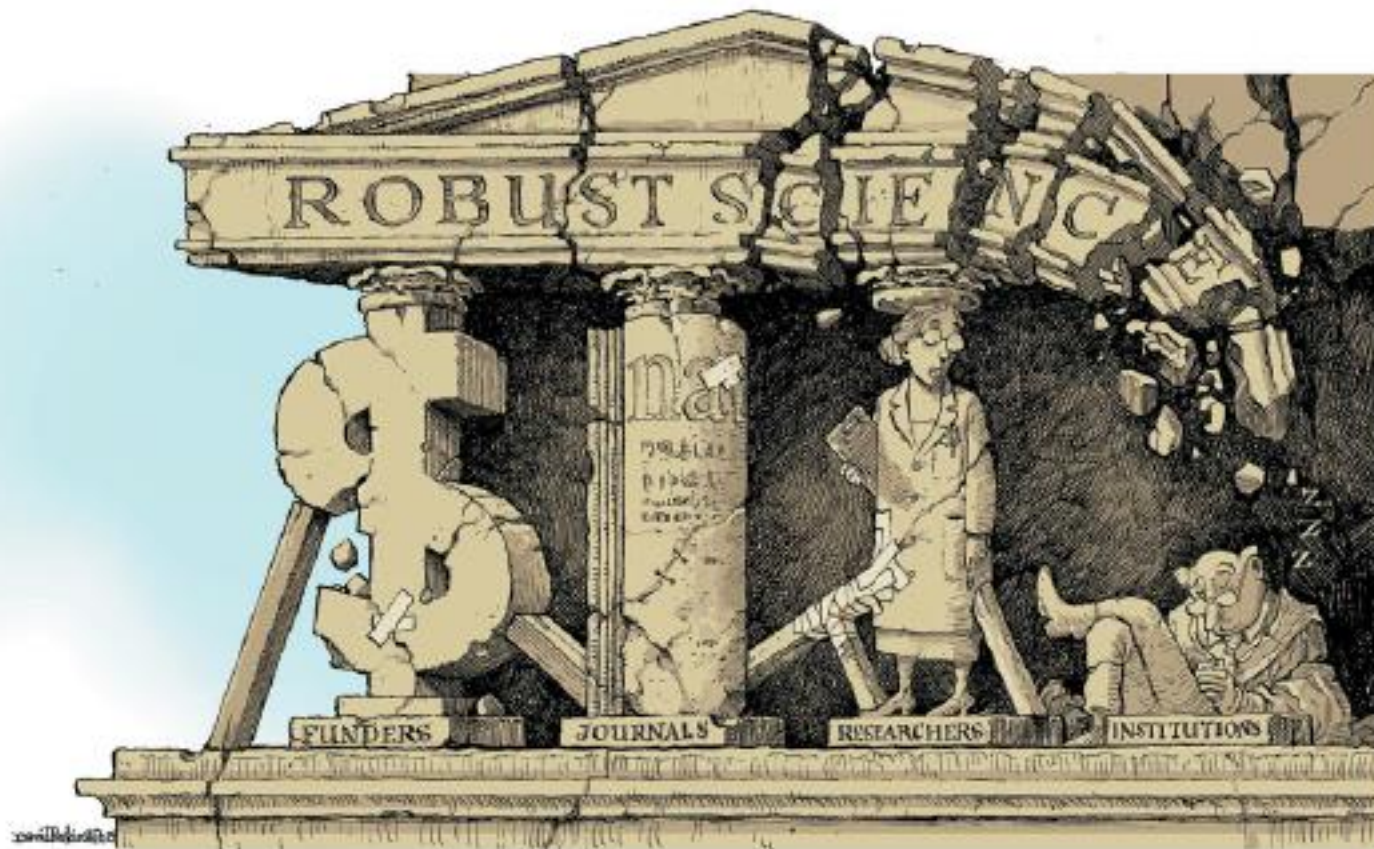
- ▶ Cost
- ▶ Time
- ▶ Attention of investigator
- ▶ Pressures to publish
- ▶ Difficulty publishing negative findings

Reproducibility Issues in Research with Animals and Animal Models: Workshop Brief

- ▶ National Academies of Sciences, Engineering, Medicine
- ▶ October, 2015
- ▶ **85% of research investment/resources is ultimately “wasted”**
 - ▶ Chalmers & Glasziou, Lancet, 374: 86, 2009.

Major Issues

- ▶ Randomization
- ▶ **BLINDED EVALUATION OF RESULTS**
- ▶ Poor protocols and documentation
- ▶ Poor utility of information
- ▶ Statistical power and outcome misinterpretations
- ▶ Lack of consideration of prior evidence
- ▶ Subjective, non-standardized definitions



Institutions must do their part for reproducibility

Tie funding to verified good institutional practice, and robust science will shoot up the agenda, say C. Glenn Begley, Alastair M. Buchan and Ulrich Dirnagl.

Good Institutional Practice (GIP)

- ▶ Routine discussion of research methods
- ▶ Reporting systems
- ▶ Training and standard
- ▶ Records and quality management
- ▶ Appropriate incentive and evaluation systems



Six red flags for suspect work

C. Glenn Begley explains how to recognize the preclinical papers in which the data won't stand up.

Red Flags for Suspect Work

- ▶ Were experiments performed blinded?
- ▶ Were basic experiments repeated?
- ▶ Were all the results presented?
- ▶ Were there positive and negative controls?
- ▶ Were reagents validated?
- ▶ Were statistical tests appropriate?

A Manifesto For Reproducible Science

- ▶ Munafò et al.
- ▶ Nature Human Behavior, Vol. 1, 2017, Article #0021

Solutions (1)

- ▶ **Methods:**
 - ▶ Protecting against cognitive biases
 - ▶ Improving methodological training
 - ▶ Independent methodological support
 - ▶ Collaboration and team science
- ▶ **Reporting and Dissemination:**
 - ▶ Promoting study pre-registration
 - ▶ Improve the quality of reporting
 - ▶ Protecting against conflicts of interest

Solutions (2)

- ▶ Reproducibility: Encouraging transparency in science
- ▶ Education: Diversifying peer review
- ▶ Incentives: Rewarding open and reproducible practices

Science is Valid
Only if It Is
Reproducible.