Rigor and what?
Making sense of the new NIH guidelines
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Occupational and Environmental Health
Overview

- Background
  - Cell culture case study
  - A personal example
- NIH New Grant Submission Guidelines
  - SIGNIFICANCE
    - Scientific Premise
  - APPROACH
    - Scientific Rigor
    - Incorporation of relevant biological variables
  - AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES
ICLAC Case Study: RGC–5

- NEED FOR A CURE: Vision loss due to glaucoma, a leading cause of blindness
- Primary cells only of limited use
- RGC–5: the first transformed cell line from rat retinal ganglion cells (2001)
- Van Bergen et al. (2009) reported RGC–5 to be of mouse origin; confirmed by Yorio and others (i.e., RGC–5 = mouse 661W cells)

http://iclac.org/case-studies/rgc-5/
ICLAC Case Study: RGC–5 (cont.)

- 2001 to 2013: 236 articles using RGC–5
- 130 (out of 236) articles published after Van Bergen et al. (2009)
- After retraction of original article: 33 articles published using RGC–5
One Outcome: Molecular Vision

“New manuscripts containing data derived from RGC-5 cells will be editorially rejected without review.”
And a personal example...

Scientific Premise:

- PCBs are metabolized by CYP2B1 to OH–PCBs
- OH–PCBs are RyR–active (= neurotoxic)
- Nicotine induces CYP2B1 in rats

Hypothesis: Nicotine increases the metabolism of PCBs to OH–PCBs in the brain due to induction of CYP2B1
CYP2B1 mRNA and CYP2B protein were not detected in brain tissues, irrespective of treatment.
Key Message

Use of incorrect key resources:

- Costs $$$
- Pushes research off course

http://iclac.org/case-studies/rgc-5/
Stages of waste in the production and reporting of research evidence relevant to clinicians and patients

Questions relevant to clinicians and patients?
- Low priority questions addressed
- Important outcomes not assessed
- Clinicians and patients not involved in setting research agendas

Appropriate design and methods?
- Over 50% of studies designed without reference to systematic reviews of existing evidence
- Over 50% of studies fail to take adequate steps to reduce biases—e.g., uncontrolled treatment allocation

Accessible full publication?
- Over 50% of studies never published in full
- Biased under-reporting of studies with disappointing results

Unbiased and usable report?
- Over 30% of trial interventions not sufficiently described
- Over 50% of planned study outcomes not reported
- Most new research not interpreted in the context of systematic assessment of other relevant evidence

Research waste
“Full publication of results initially presented in abstracts”

Only 63% of results from abstracts describing randomized or controlled clinical trials are published in full.

'Positive' results were more frequently published than not 'positive' results.

Quoted from: Cochrane database of systematic reviews (Online) Issue 2, 2007, Pages MR000005
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Old NIH Proposal Structure

- SPECIFIC AIMS (1 page)
- RESEARCH STRATEGY (12 pages)
  - Progress Report (only renewal)
  - Significance
  - Innovation
  - Approach
    - Each Specific Aim
      - Introduction
      - Justification & Preliminary Data
      - Research Design
      - Expected Outcomes
      - Anticipated Problems and Alternative Approaches
  - Timetable (optional)
  - Future Directions (optional)

CYP2B1 mRNA and CYP2B protein were not detected in brain tissues
The **scientific premise** for an application is the research that is used to form the **basis** for the proposed research question(s). …

… this consideration … could include attention to the **rigor** of the previous experimental designs, as well as the incorporation of relevant biological variables and authentication of key resources.
Research Strategy: “Significance”

- Describe the **scientific premise** (= scientific foundation)

- Consideration of strengths and weaknesses:
  - Published research and
  - crucial preliminary data

CYP2B1 mRNA and CYP2B protein were not detected in brain tissues.

SPECIFIC AIMS (1 page)

RESEARCH STRATEGY (12 pages)
- Progress Report (only renewal)
- Significance
  - Overall Scientific Premise: Literature and Preliminary Data
  - Scientific Premise for each Specific Aim: Literature and Preliminary Data
- Innovation
- Approach
  - Each Specific Aim
    - Introduction
    - Research Design
    - Expected Outcomes
    - Anticipated Problems and Alternative Approaches
- Timetable (optional)
- Future Directions (optional)
What does NIH mean with “strength and weaknesses”?

- **Rigor** of previous experimental design
  - Statistical power?
  - Blinded studies?
  - ...

- Incorporation of relevant **biological variables**
  - Sex?
  - ...

- **Authentication of key resources**
  - Authentication of cell lines
  - Characterization of chemicals
  - ...
A call for transparent reporting to optimize the predictive value of preclinical research


The US National Institute of Neurological Disorders and Stroke convened major stakeholders in June 2012 to discuss how to improve the methodological reporting of animal studies in grant applications and publications. The main workshop recommendation is that at a minimum studies should report on sample-size estimation, whether and how animals were randomized, whether investigators were blind to the treatment, and the handling of data. We recognize that achieving a meaningful improvement in the quality of reporting will require a concerted effort by investigators, reviewers, funding agencies and journal editors. Requiring better reporting of animal studies will raise awareness of the importance of rigorous study design to accelerate scientific progress.
Core Reporting Standards

- Randomization
- Blinding
- Sample-size estimation
- Data handling

BOX 1
A core set of reporting standards for rigorous study design

Randomization
- Animals should be assigned randomly to the various experimental groups, and the method of randomization reported.
- Data should be collected and processed randomly or appropriately blocked.

Blinding
- Allocation concealment: the investigator should be unaware of the group to which the next animal taken from a cage will be allocated.
- Blinded conduct of the experiment: animal caretakers and investigators conducting the experiments should be blinded to the allocation sequence.
- Blinded assessment of outcome: investigators assessing, measuring or quantifying experimental outcomes should be blinded to the intervention.

Sample-size estimation
- An appropriate sample size should be computed when the study is being designed and the statistical method of computation reported.
- Statistical methods that take into account multiple evaluations of the data should be used when an interim evaluation is carried out.

Data handling
- Rules for stopping data collection should be defined in advance.
- Criteria for inclusion and exclusion of data should be established prospectively.
- How outliers will be defined and handled should be decided when the experiment is being designed, and any data removed before analysis should be reported.
- The primary end point should be prospectively selected. If multiple end points are to be assessed, then appropriate statistical corrections should be applied.
- Investigators should report on data missing because of attrition or exclusion.
- Pseudoreplicate issues need to be considered during study design and analysis.
- Investigators should report how often a particular experiment was performed and whether results were substantiated by repetition under a range of conditions.
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Research Strategy: “Approach”

- How will the experimental design/methods achieve robust and unbiased results?

- Relevant biological variables must be factored into research design/analyses:
  - Vertebrate animals
  - Humans

- Biological variables:
  - Sex
  - Weight
  - Age
  - Health conditions

Biological Variables: 4 Cs In Studying Sex To Strengthen Science

- **Consider**
  - Studies should consider sex
  - Explain why sex does not need to be considered

- **Collect**
  - Tabulate sex data

- **Characterize**
  - Analyze sex based data

- **Communicate**
  - Report & publish sex-based data
Aim 3: Male and female mice will be randomly allocated to experimental groups at age 3 months. At this age the accumulation of CUG repeat RNA, sequestration of MBNL1, splicing defects, and myotonia are fully developed. The compound will be administered at 3 doses (25%, 50%, and 100% of the MTD) for 4 weeks, compared to vehicle-treated controls. IP administration will be used unless biodistribution studies indicate a clear preference for the IV route. A group size of \( n = 10 \) (5 males, 5 females) will provide 90% power to detect a 22% reduction of the CUG repeat RNA in quadriceps muscle by qRT–PCR (ANOVA, \( \alpha \) set at 0.05). The treatment assignment will be blinded to investigators who participate in drug administration and endpoint analyses. This laboratory has previous experience with randomized allocation and blinded analysis using this mouse model [refs]. Their results showed good reproducibility when replicated by investigators in the pharmaceutical industry [ref].
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New: Authentication of Key Biological and/or Chemical Resources Attachment

Key biological and/or chemical resources may or may not be generated with NIH funds and:

1) **differ** from laboratory to laboratory or over time;

2) have qualities and/or qualifications that could **influence the research data**

3) are **integral** to the proposed research

Examples of Key Biological and/or Chemical Resources

- Cell lines
- Antibodies
- Other biologics (e.g., transgenic animals)
- Specialty chemicals

Standard laboratory reagents do not need to be included:
- Buffers
- Common biologicals or chemicals
Authors must **declare what cell lines were used**. Describing sources of cell lines indicates their origin and allows for the research to be reproduced.

Manuscripts ... are **checked at initial submission**. Those that do not meet the requirements ... will be rejected. Issues ... discovered after publication may **lead to a correction or retraction**.

Editors and reviewers **should evaluate** cell line information during peer review ....
State **when and where** they obtained the cells, giving the **date** and the **source** who provided the cells

For **established cell lines**:

- A **reference** to the published article that first described the cell line; AND/OR

- The cell line repository or company the cell line was obtained from, the **catalogue number**, and whether the cell line was obtained directly from the repository/company or from **another laboratory**
PLOSone Submission Guidelines: Cell Lines (cont.)

- Check the ICLAC Database of Cross-contaminated or Misidentified Cell Lines

- Cell line authentication is recommended:
  - Karyotyping
  - Isozyme analysis
  - Short tandem repeats (STR) analysis

- Cell line authentication may be required during peer review or after publication.
ICLAC Cell Line Checklist for Manuscripts and Grant Application

ICLAC
INTERNATIONAL CELL LINE AUTHENTICATION COMMITTEE

Cell Line Checklist for Manuscripts and Grant Applications

- **QUALITY**
  - **YES** Check the database of misidentified cell lines
  - **YES** Authenticate your sample
  - **YES** Make your STR profile available

Useful Resources
- ICLAC Database of Cross-Contaminated or Misidentified Cell Lines
- Advice to Scientists: Incorporating Authentication into Everyday Culture Practice
- Cell Line Checklist for Manuscripts and Grant Applications
- Guide to Human Cell Line Authentication
- Match Criteria Worksheet for Human Cell Line Authentication
- Naming a Cell Line
- Resources for Authentication Testing Survey
- ICLAC Terms of Reference

Download the latest version of the ICLAC Cell Line Checklist [here](http://iclac.org/resources/cell-line-checklist/) (updated 9 May 2014).
Information Regarding Antibodies

- Name of each antibody
- Description of whether it is monoclonal or polyclonal
- Host species
- Commercial supplier or source laboratory
- Catalogue or clone number; batch number (if known)
- Antigen(s) used to raise the antibody
- **Stable public identifier** (Antibody Registry; eagle-i repository)
Experimental Details Regarding Antibodies

- Final antibody concentration or dilution
- Reference to validation study

or

- How was the antibody validated for:
  - applications
  - species used
Antibody Resource Identifiability Across Disciplines (n = 703 antibodies)

- Common issues
  - Lack of catalog number
  - Lack of reference to the immunogen

- Recommended citation of antibody:
  
  Vendor, Cat #, RRID

  Example: (Cell Sciences Cat# PA0871BT, RRID:AB_10052953)

Authentication of Key Chemical Resources

- Gas or liquid chromatographic analysis

- \textit{isrp} Synthesis Core standard:
  - Full characterization according the JOC
  - Accurate Mass Determination or Elemental Analysis
  - Purity determination: GC–FID and/or GC–MS
  - X–ray crystal structure if possible
  - Provide characterization in publication