Modello animale, suo significato, limiti applicativi e modellistica predittiva: focus sulla “robustezza” del dato sperimentale

Laura Calzà

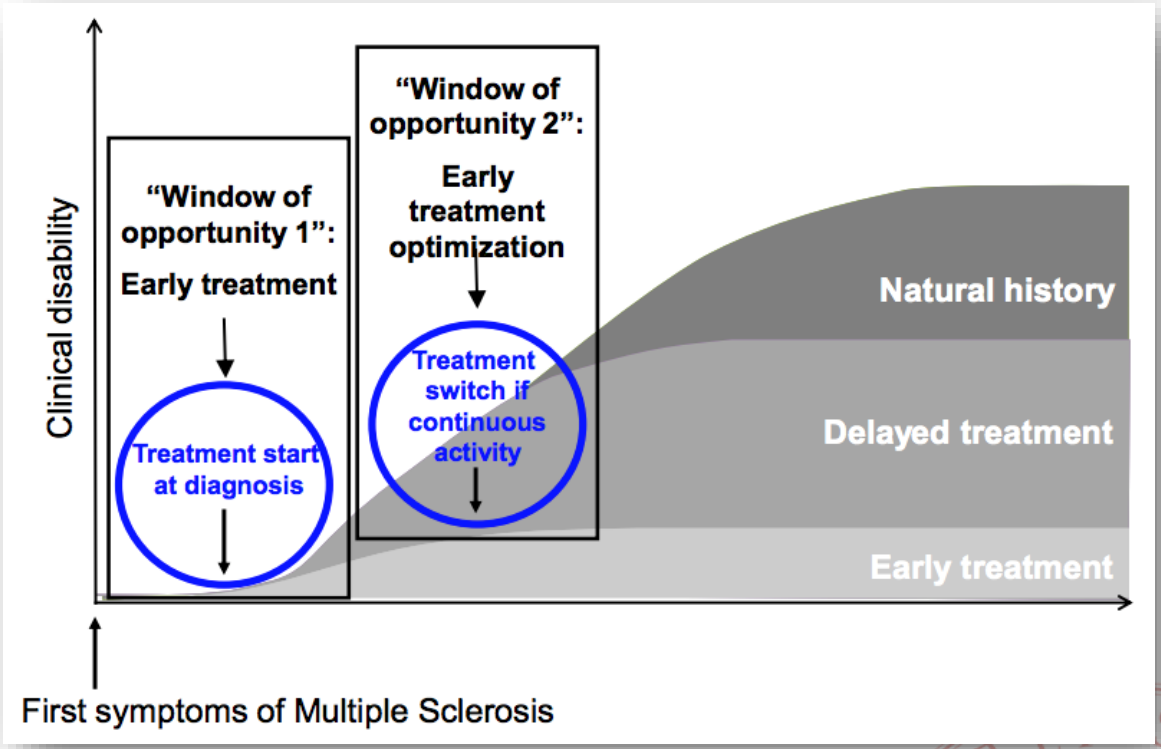
CIRI-SDV e FaBit, Università di Bologna; IRET Foundation, Ozzano Emilia

laura.calza@unibo.it

EAE for multiple sclerosis drug treatment

Immunomodulatory therapies
*1971: first positive result in **EAE***
*2009: Copaxone **FDA** approval*

- **Interferon B** (Avonex, Rebif (interferone beta 1a); Betaferon (interferone beta 1b))
- **Natalizumab** (Tysabri, Elan & Biogen Idec): monoclonal antibody against the α4β1 integrin
- **FTY720** (fingolimod, Novartis Pharmaceuticals): acts by limiting egress of lymphocytes out of secondary lymphoid tissues
- **Glatiramer acetate**, GA (Copaxone, Teva Pharmaceuticals), "bystander suppression"
- **Alemtuzumab** (Campath, Genzyme Corp.)

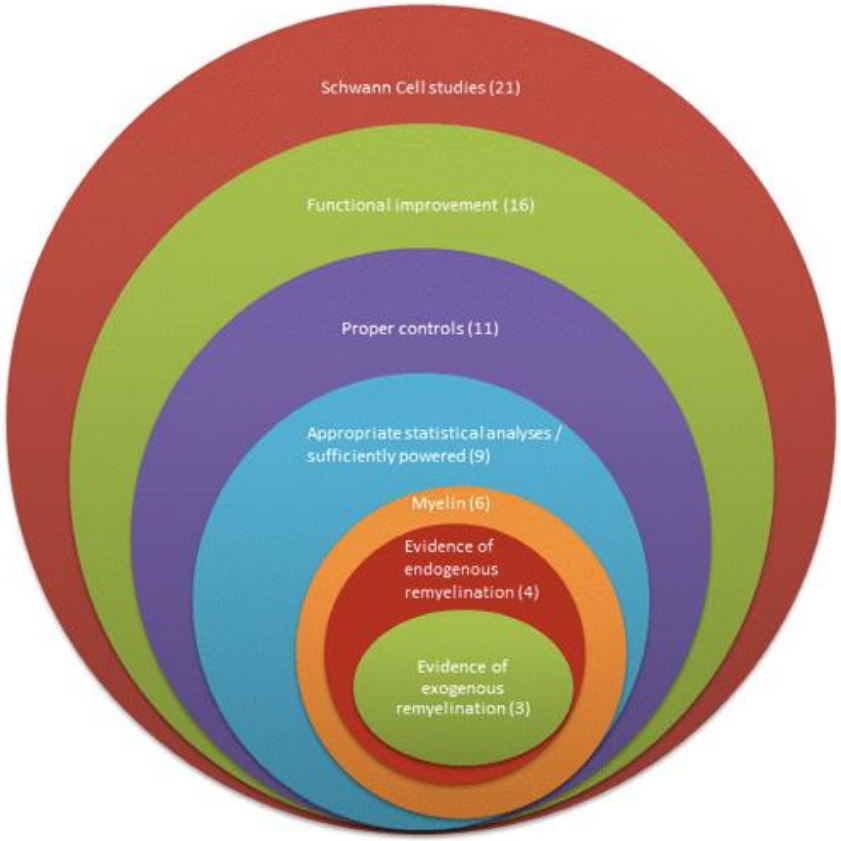


Ziemssen et al., 2016

Due to incomplete evaluation and lack of replication, full consensus regarding the potential for cell therapy in the field of SCI has yet to be achieved….

In the context of remyelination, none of these clinical trials are fully aligned with the preclinical data

Mayers et al, Exp neurol, 2016



21

Schwann Cells

Functional improvement

Proper controls

Appropriate statistical analysis/sufficient power

Myelin

Endogenous remyelination

Exogenous remyelination

3

- Premesse: da non dimenticare
- Modello animale: quale obiettivo?
- “Robustezza” del dato sperimentale: **prerequisito disatteso?**
- Che fare: formazione
  laboratorio
  istituzioni
  comunità scientifica

Download ARRIVE guidelines

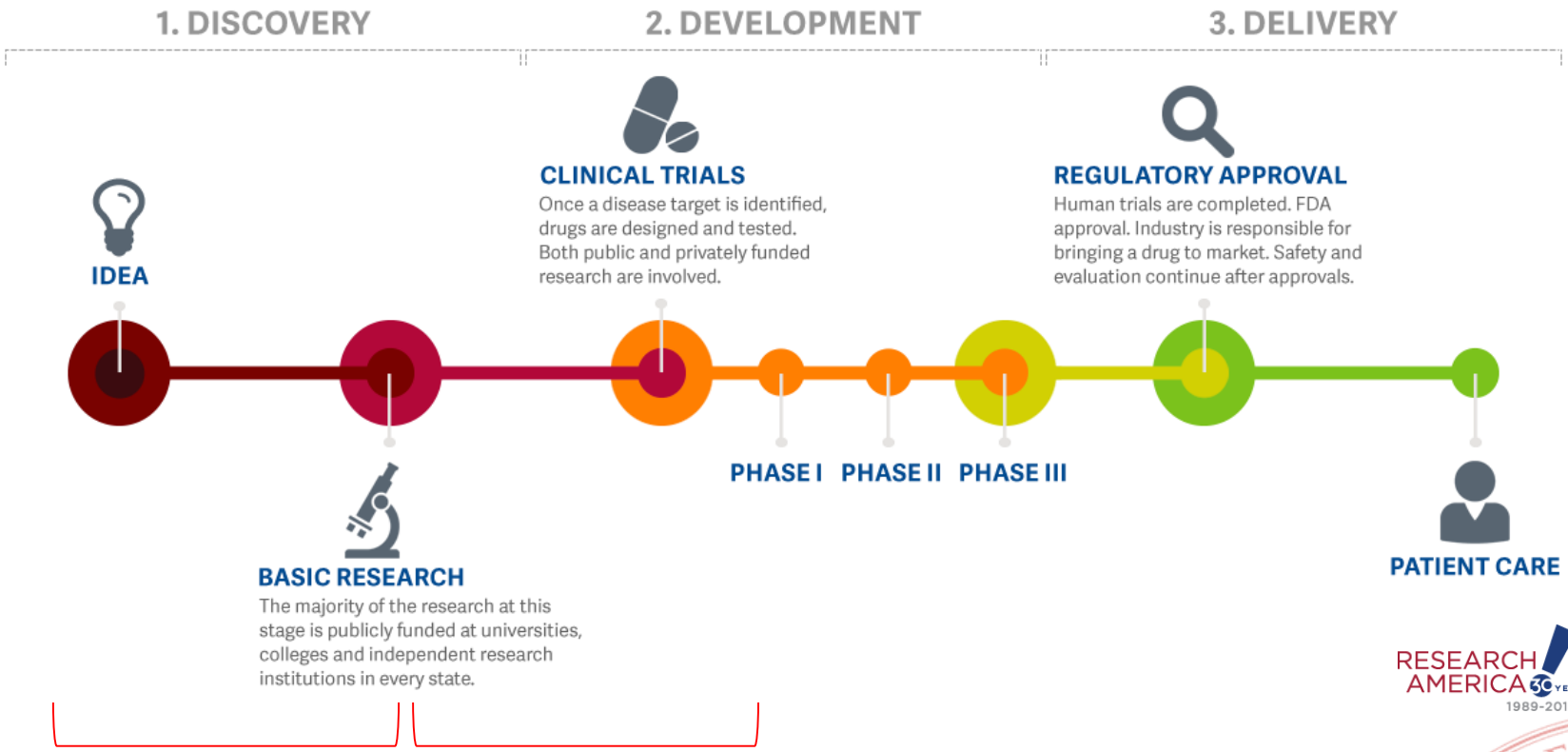
La sperimentazione animale trova una sua giustificazione solo all'interno di un preciso contesto (Direttiva n. 2010/63/UE, Decreto legislativo 4 marzo 2014, n. 26)

- massima protezione degli animali
- l'impiego di animali vivi continua ad essere necessario per tutelare la **salute** umana e animale e l'ambiente
- ogni utilizzo di un animale sia attentamente valutato considerando la validità, l'utilità e la pertinenza scientifica o educativa del risultato che si prevede di ottenere da tale utilizzo

Evidence-based medicine: three common criteria for judging evidence:

- Relevance: evidence has to constitute information for (or against) some proposition
- Sufficiency: information must meet the criteria of corroboration with other pieces of information on the same topic
- Veracity: process of gathering has been free from distortion and as far as possible uncontaminated by vested interests

Modello animale: quale obiettivo?

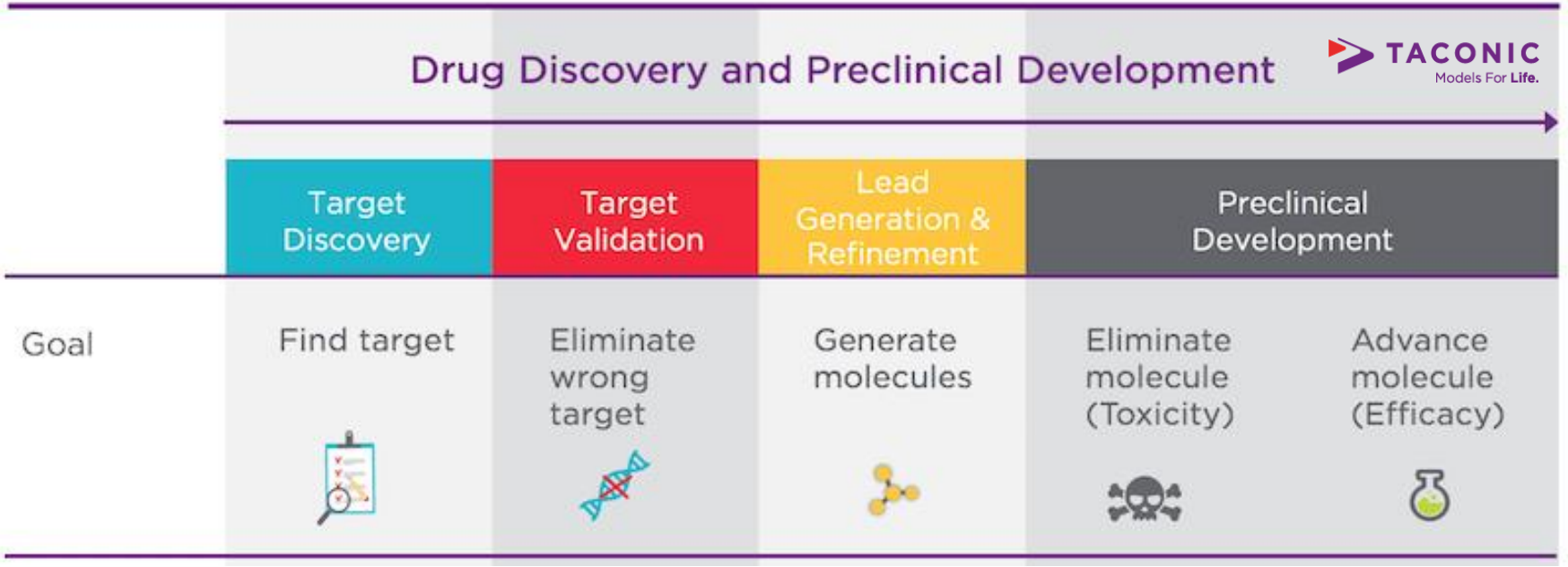


Mode 1
of knowledge production
"**basic** science"

Mode 2
of knowledge production
"**applied** and **translational** research"

Gibbons et al, 1994

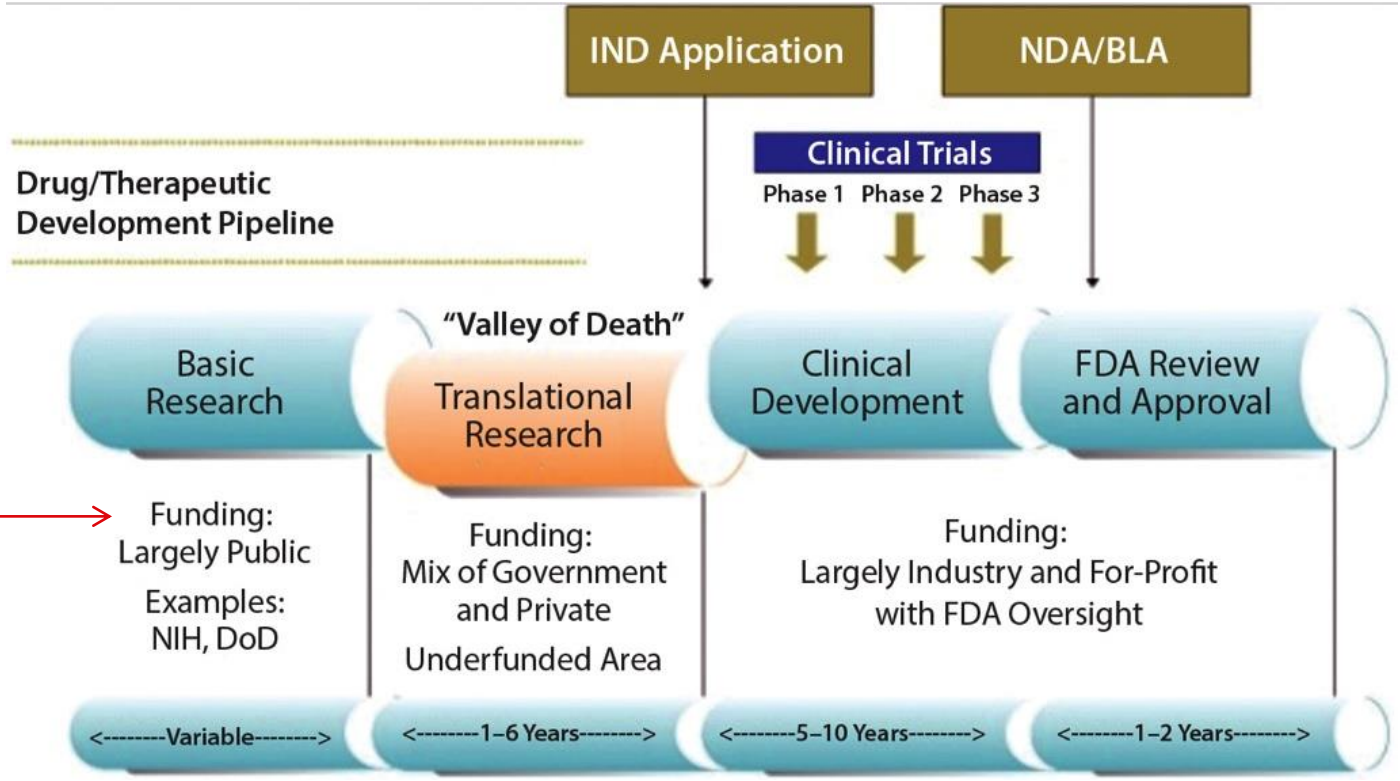
Modello animale nella "discovery phase"



Mode 1
Basic science

Mode 2
Applied and Translational Research

Drug development pipeline



bioprocessintl.com

Science Translational Medicine AAAS

Science Translational Medicine (2009)

Integrating Medicine and Science

What is Translational Medicine?

Often described as an effort to carry scientific knowledge "from bench to bedside," translational medicine builds on basic research advances – studies of biological processes using cell cultures, for example, or animal models – and uses them to develop new therapies or medical procedures.

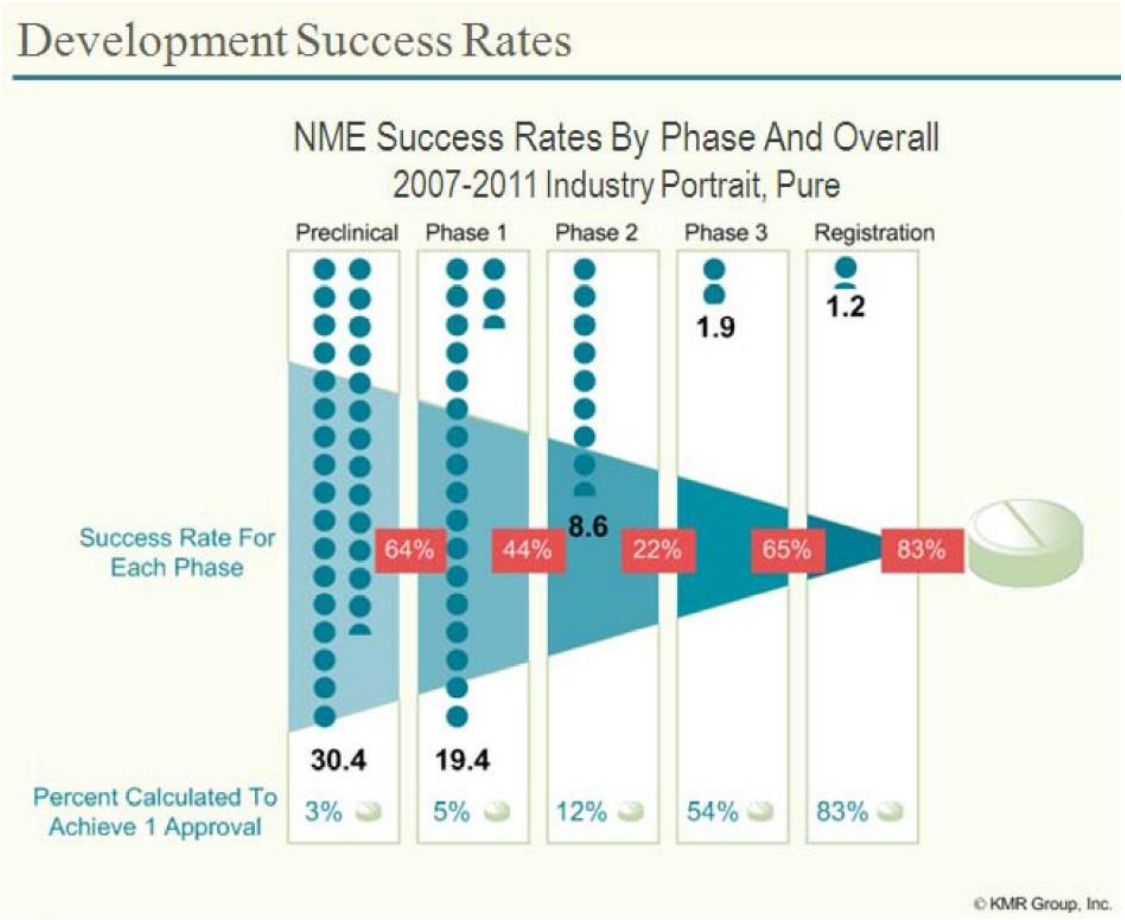
Translational medicine is becoming ever-more interdisciplinary. For example, researchers need new computational approaches to deal with the large amounts of data pouring in from genomics and other fields, and as new advances in physics and materials science offer new approaches to study or diagnose medical conditions.

AIC: Autorizzazione all'immissione in commercio di un farmaco.

Viene concessa dall'AIFA dopo che un gruppo di esperti ne ha valutato la **sicurezza** e **l'efficacia**. Costituisce la "carta di identità" del medicinale poiché in essa sono indicati le caratteristiche essenziali che lo identificano.

Modello animale: utile o inutile??

Lovell-Badge, MRC National Institute for Medical Research, London



Preclinical (including animal experiments) remove 36% of the potential drugs from moving onto the next stage

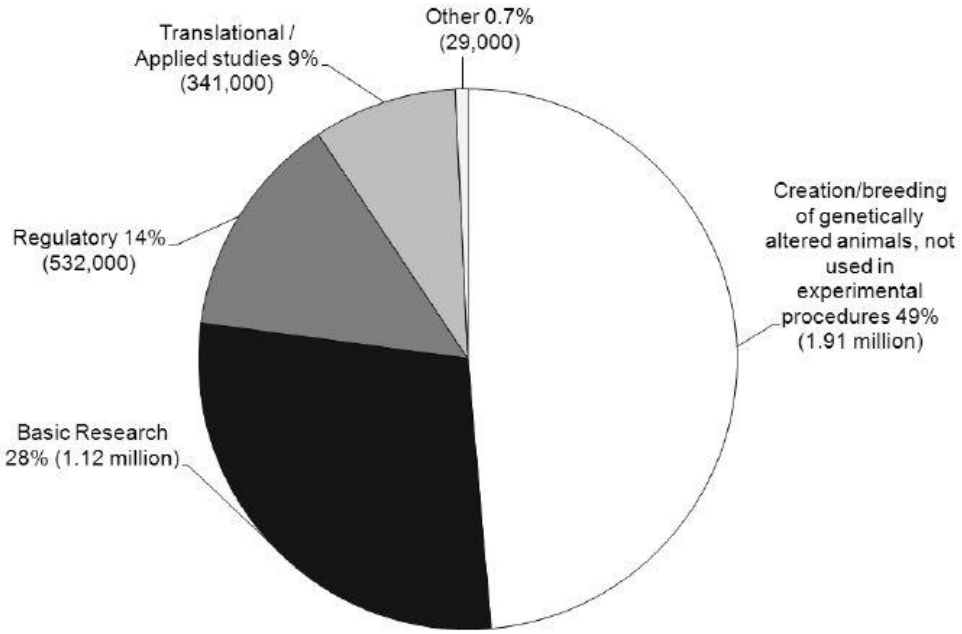
FDA (2004):

"Currently, nine out of ten experimental drugs fail in clinical studies because we cannot accurately predict how they will behave in people based on laboratory and animal studies"

… a new medicinal compound entering Phase 1 testing, often representing the culmination of upwards of a decade of preclinical screening and evaluation, is estimated to have only an 8 percent chance of reaching the market"

Modello animale: quale obiettivo?

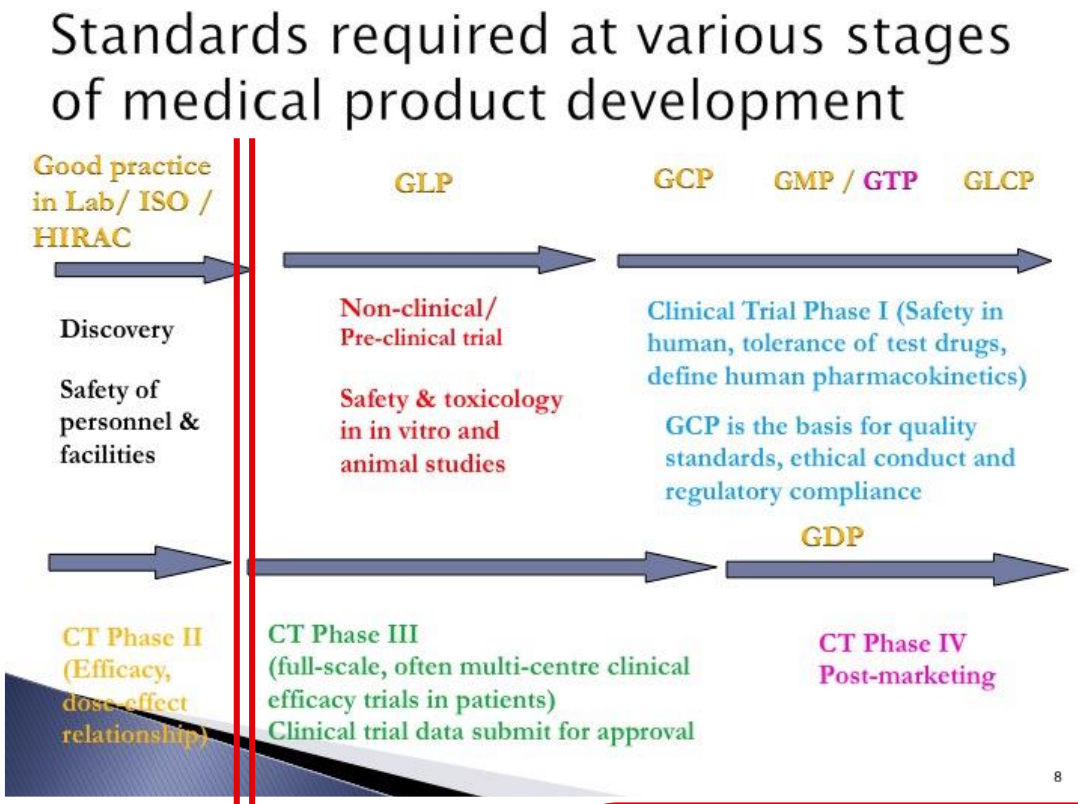
- "curiosity-drived" experiment: per produrre conoscenza
- PoC: per confermare un'idea
- Efficacia preclinica: per IND application (Investigational New Drug)
- Sicurezza preclinica (GLP): per AIC



2016, UK, gov:

- 9% Applied research
- 14% Regulatory testing
- 28% Basic biological and medical research
- 49% Breeding of laboratory animals
  (genetically altered animals, mostly for research and developing new treatments)

“translational gap” o “valley of death”



“Buone pratiche” su base individuale/Ente

GLP
GCP
GMP
GxP

Leggi degli Stati membri OECD *(Organisation for Economic Co-operation and Development)*

Robustness of preclinical R&D

PERSPECTIVE

doi:10.1038/nature11556

A call for transparent reporting to optimize the predictive value of preclinical research

Story C. Landis[1], Susan G. Amara[2], Khusru Asadullah[3], Chris P. Austin[4], Robi Blumenstein[5], Eileen W. Bradley[6], Ronald G. Crystal[7], Robert B. Darnell[8], Robert J. Ferrante[9], Howard Fillit[10], Robert Finkelstein[1], Marc Fisher[11], Howard E. Gendelman[12], Robert M. Golub[13], John L. Goudreau[14], Robert A. Gross[15], Amelie K. Gubitz[1], Sharon E. Hesterlee[16], David W. Howells[17], John Huguenard[18], Katrina Kelner[19], Walter Koroshetz[1], Dimitri Krainc[20], Stanley E. Lazic[21], Michael S. Levine[22], Malcolm R. Macleod[23], John M. McCall[24], Richard T. Moxley III[25], Kalyani Narasimhan[26], Linda J. Noble[27], Steve Perrin[28], John D. Porter[1], Oswald Steward[29], Ellis Unger[30], Ursula Utz[1] & Shai D. Silberberg[1]

11 OCTOBER 2012 | VOL 490 | NATURE | 187

PLOS | BIOLOGY

COMMUNITY PAGE

Meta-research: Evaluation and Improvement of Research Methods and Practices

John P. A. Ioannidis*, Daniele Fanelli, Debbie Drake Dunne, Steven N. Goodman

Meta-Research Innovation Center at Stanford (METRICS), Stanford University, Stanford, California, United States of America

eNeuro 2016

Commentary

History, Teaching, and Public Awareness

Statistical Rigor and the Perils of Chance

Katherine S. Button[1]

DOI:http://dx.doi.org/10.1523/ENEURO.0030-16.2016

[1]Department of Psychology, University of Bath, Bath BA2 7AY, United Kingdom

OPEN ACCESS Freely available online

PLOS | BIOLOGY

Evaluation of Excess Significance Bias in Animal Studies of Neurological Diseases

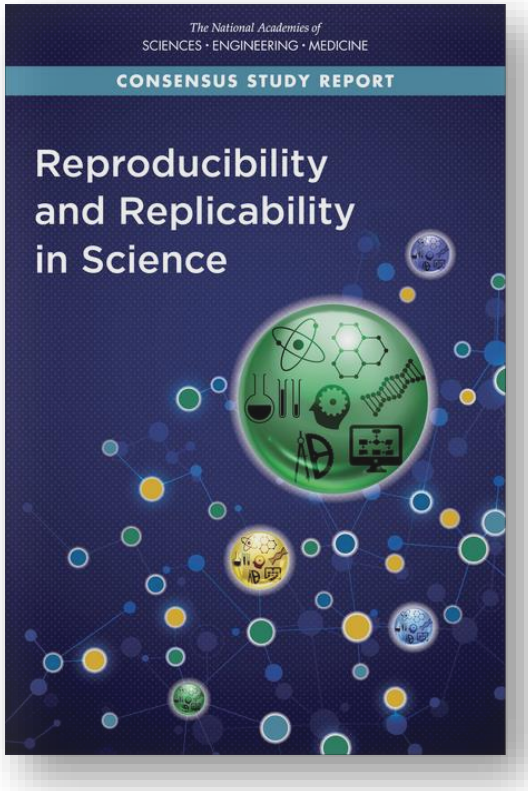
Konstantinos K. Tsilidis[1☯], Orestis A. Panagiotou[1☯], Emily S. Sena[2,3], Eleni Aretouli[4,5], Evangelos Evangelou[1], David W. Howells[3], Rustam Al-Shahi Salman[2], Malcolm R. Macleod[2], John P. A. Ioannidis[6]*

ANALYSIS

Power failure: why small sample size undermines the reliability of neuroscience

Katherine S. Button[1,2], John P. A. Ioannidis[3], Claire Mokrysz[1], Brian A. Nosek[4], Jonathan Flint[5], Emma S. J. Robinson[6] and Marcus R. Munafò[1]

Reproducibility and replicability in science



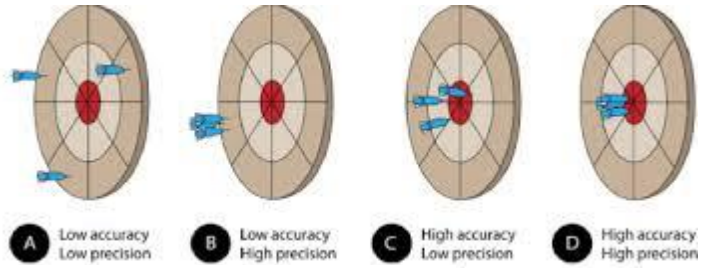
Consensus Report, 2019

Reproducibility means obtaining consistent computational results using the same input data, computational steps, methods, code, and conditions of analysis

Replicability means obtaining consistent results across studies aimed at answering the same scientific question, each of which has obtained its own data

Accuracy

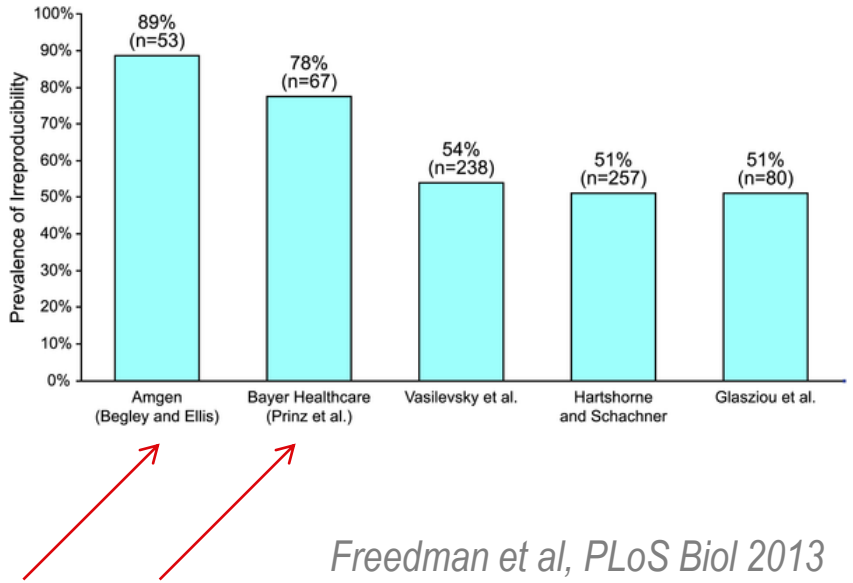
Precision



The National Academies of SCIENCES ENGINEERING MEDICINE

ELSEVIER
European Neuropsychopharmacology
Volume 25, Issue 10, October 2015, Pages 1803–1807

Studies reporting the prevalence of irreproducibility

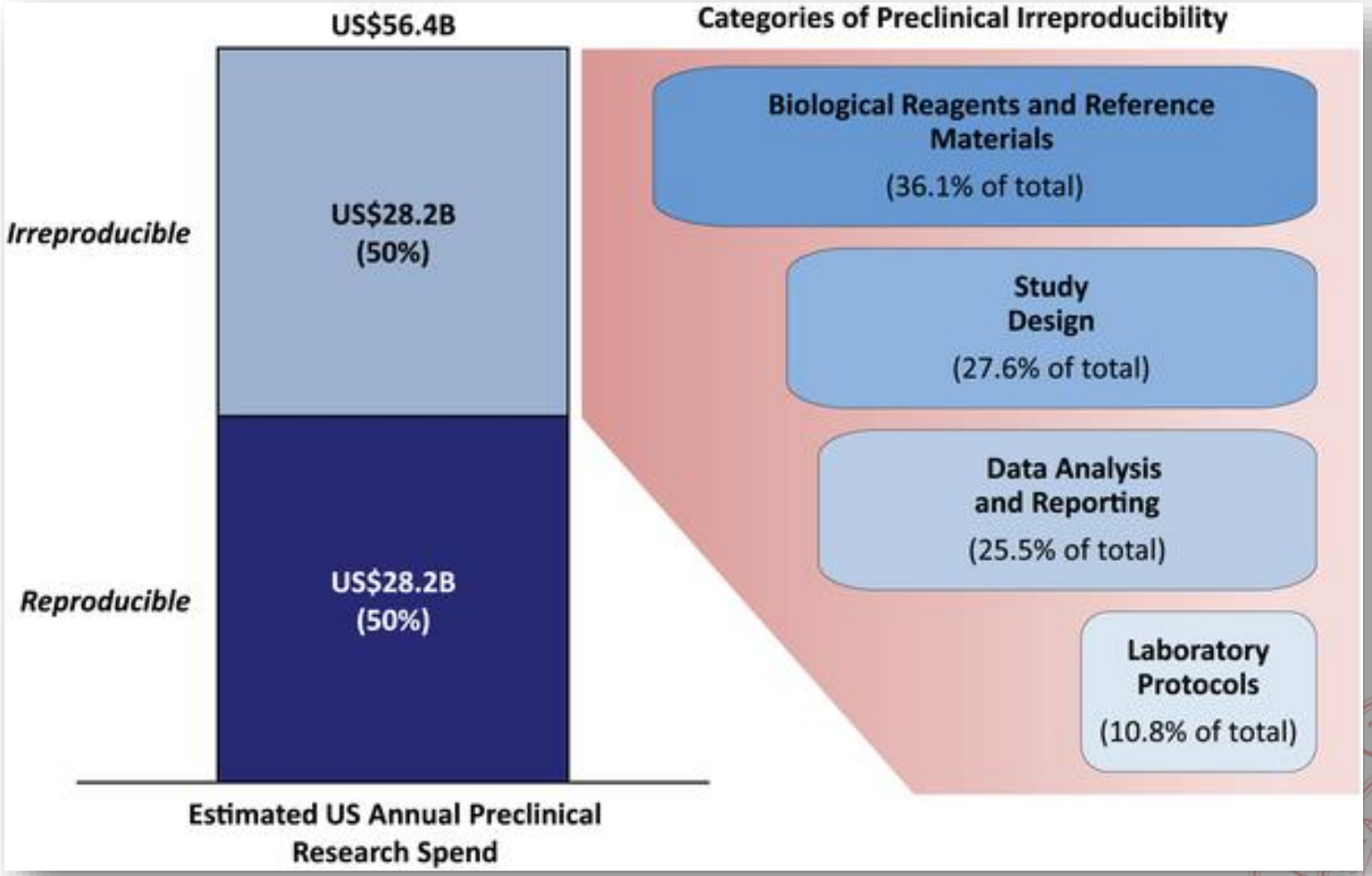


Freedman et al, PLoS Biol 2013

Table 1.
Challenges for data reproducibility and quality.

Frequency of occurrence	Outcome	Source
1.97%	Of scientists self-reported data fabrication, falsification or modification at least once	Fanelli, 2009
31%	Of animal studies on neurological disorders showed evidence for excess statistical significance, suggesting bias	Tsilidis et al., 2013
34%	Of scientists self-reported questionable research practices	Fanelli, 2009
54%	Of resources published were not uniquely identifiable in published biomedical studies, making replication difficult	Vasilevsky et al., 2013
55%	Of MD Anderson Cancer Center scientists experienced at least one incidence of being unable to reproduce published data	Mobley et al., 2013
57%	Of neuroscience studies found to have low statistical power (≤30%), hence low reliability	Button et al., 2013
57%	Of internal study protocols were amended after statistical review at Astra Zeneca – would figures from published studies be comparable or possibly even worse?	Peers et al., 2014
65%	Of published data (oncology, women's health, cardiovascular) were inconsistent with internal data at Bayer	Prinz et al., 2011
72%	Of scientists reported questionable research practices by colleagues	Fanelli, 2009
78%	Of studies in social sciences with null results remained unpublished	Franco et al., 2014
85%	Of resources have been estimated to be wasted in science	Chalmers and Glasziou, 2009
0%	Of out of more than 100 compounds previously suggested to be potential ALS drugs found active in an ASL mouse model if standardized study design was used	Perrin, 2014

Costs and categories of irreproducibility: lack of a standard and best practice framework



(Freedman et al., PlosBiol, 2015)

CellPress

Neuron

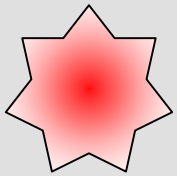
Perspective

Rigor or Mortis: Best Practices for Preclinical Research in Neuroscience

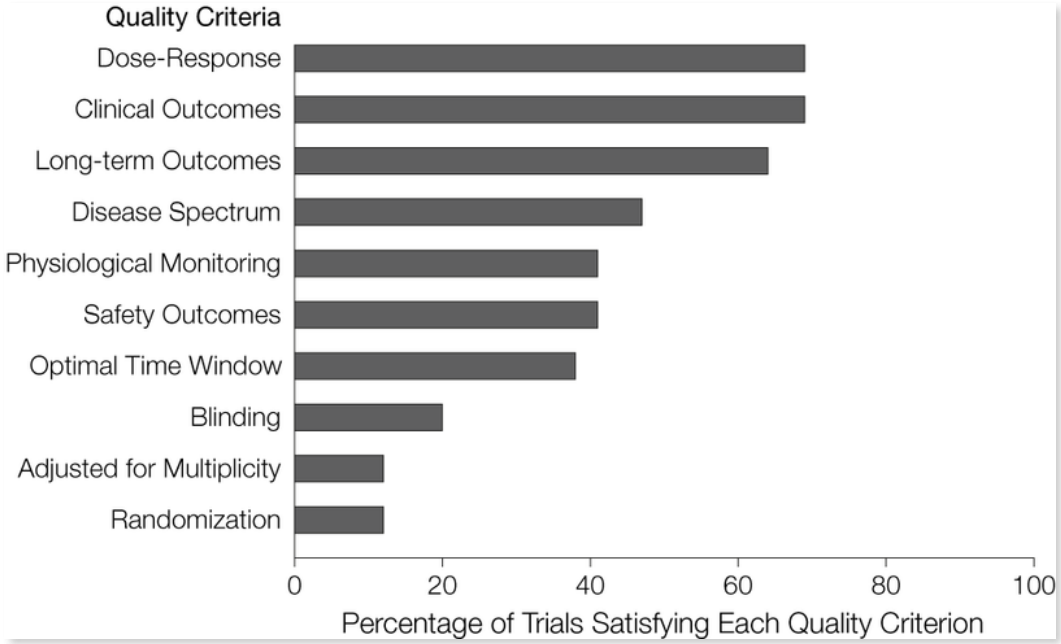
Oswald Steward[1,*] and Rita Balice-Gordon[2,*]

Table 1. A Primer of Best Practices to Enhance Rigor and Reproducibility

Topic	Best Practice	Benefits
Experimental Design	Describe experiment planning in manuscript Methods section, including: ● Power calculations (endpoint sensitivity, variability, effect size, desired level of confidence, definition and rationale for n). ● Inclusion/exclusion of data sets, description of pilot, and final data sets included in analyses. ● Random assignment to treatment groups, description of exceptions. ● Procedures to achieve blinding, exceptions to blinding, and resulting interpretive caveats. ● Details of reagents and assays sufficient to facilitate independent replication. ● Positive and negative controls.	Capture thinking in incomplete information landscape. Iterative hypothesis refinement. Deep understanding of assessments in advance of execution. Reduce testing to foregone conclusion. Optimize resource allocation and use. Create roadmap to assembling publication.

Analysis and Statistics 	Describe statistical analysis plan in manuscript Methods section, including: • Methods to test for significance. • Interim analyses, futility assessments. • Data inclusion/exclusion, attrition. • Statistical treatment of technical and biological replicates. • Test-retest approaches. • Statement of central tendency, variance, statistical test, and p value for significant and nonsignificant differences. • Descriptive statistics for groups as well as pooled values.	Enhance awareness of and reduce sources of potential unconscious bias. Minimize type 1 error.
Data Management 	Develop lab standards for indexing and maintaining information, including: • Recording of key experimental design and execution parameters. • Archiving raw data and at least one backup with appropriate frequency. • Curation of process from raw data to summary figure to conclusion.	Ensure all information supporting a conclusion can be located during and after study completion.
Resource Sharing	Include lists of resources in manuscripts that will be made available and point of contact for requests. Indicate time limit for resource availability, if any. Include budget line item to support resource sharing in funding applications. Deposit animal lines at commercial vendor within 3 months of publication. Provide raw data upon request.	Simplify sharing of reagents, protocols, raw data to facilitate replication, interpretation of data. Help distinguish lack of conceptual validation versus lack of replication. Enable meta-analyses and data basing.
Publication and Reporting	Provide comprehensive review checklist for methodology, reagents, and resource sharing. Two-stage review: if manuscript meets general journal criteria (novelty, impact, general interest), initiate second stage of review for technical merit including details relating to rigor.	Raise awareness of key metrics for determining rigor. Facilitate replication of key findings.

Methodological Quality of Animal Trials (n=76)



JAMA, 2006

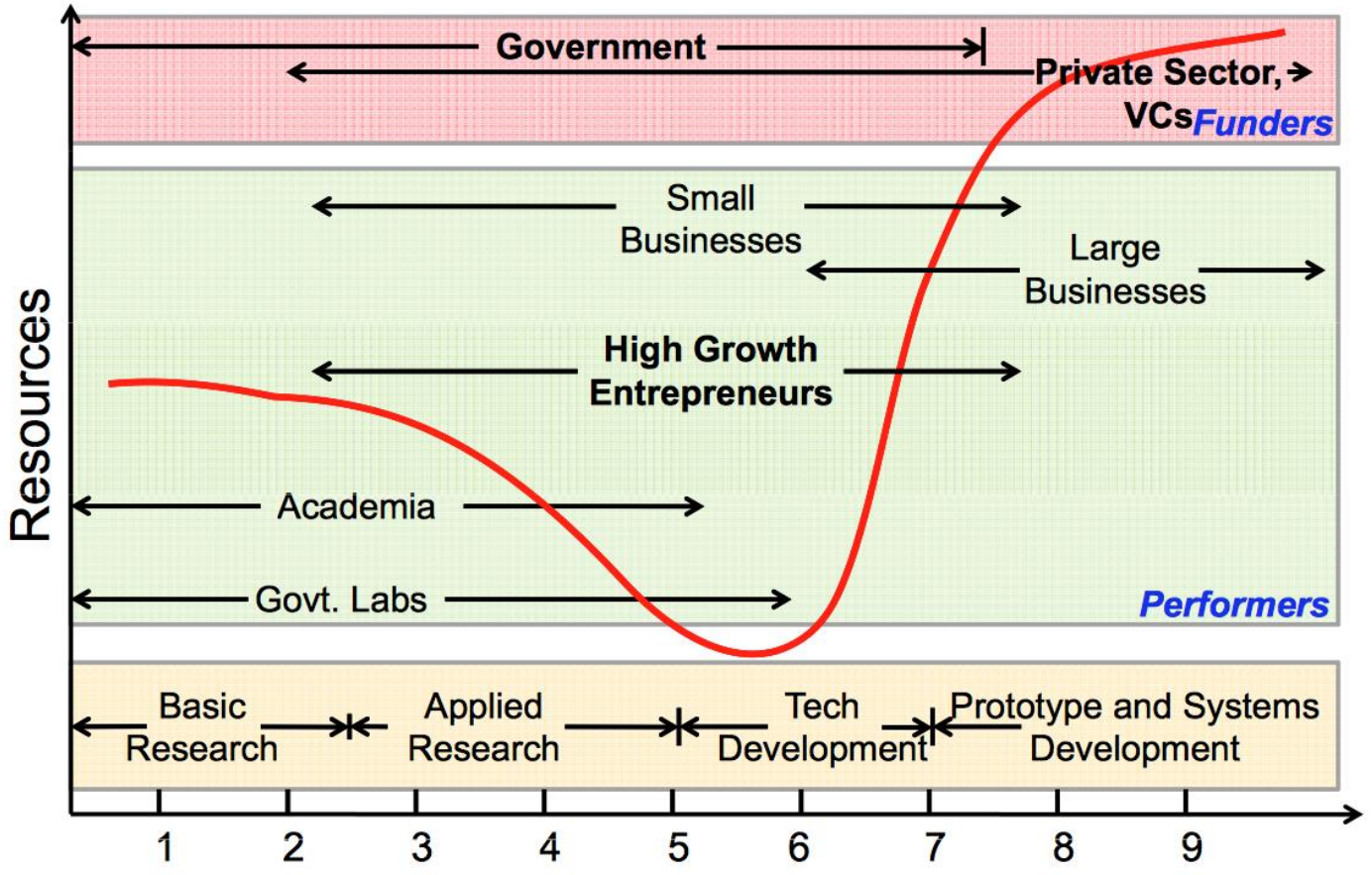
Table 1. CNS Program Portfolios in Large Pharma: 2009 versus 2014

	2009	2014
Total Programs	267	129
Abbott/AbbVie	17	10
AstraZeneca	21	7
Bristol-Myers Squibb	12	2
GlaxoSmithKline	40	14
Johnson & Johnson	18	17
Lilly	16	9
Merck/Schering-Plough	32	7
Novartis	14	15
Pfizer/Wyeth	46	15
Roche/Genentech	22	21
Sanofi/Genzyme	29	12

Total number of discovery, preclinical, and clinical drug development programs addressing neurology or psychiatry disease targets, visible from publicly available sources including SEC filings, investor briefings, and company websites. Reproduced from Choi et al. (2014).

Neuron, 2014

“Evidence” in translational perspective



Tempo:	1-15 anni
Costo:	fino a 1miliardo$
Attrito:	1:100000

SHORT COURSE 3

Optimizing Experimental Design for High-Quality Science

Organizers: Mara Dierssen, MD, PhD; Magda Giordano, PhD; Chris McBain, PhD; Charles Mobbs, PhD; John Ngai, PhD; Rae Nishi, PhD

1–5:30 p.m.

McCormick Place: N227

Contact: mpd@sfn.org

The scientific community has become increasingly concerned about issues related to data reproducibility and experimental design. Issues include, but are not limited to: bias for positive results, the "p-hacking" effect, lack of sufficient replication of experiments, pooling data from different experiments, lack of randomization and/or blinding, chance observations, data selection, group compilation, and lack of rigorous training in statistics and analysis. Attendees will learn experimental and analytical design elements that are crucial for the interpretation of neuroscience research results, such as methodological parameters that can introduce bias, influence robustness, or may be subject to biological variability, and the biological and sociological underpinnings of scientific bias. Existing policies on data deposition and presentation will additionally be covered. Lectures will be interspersed with small group discussion opportunities to allow ample time for the examination of case studies.

Che fare???

formazione

SHORT COURSE 3

Optimizing Experimental Design for High-Quality Science

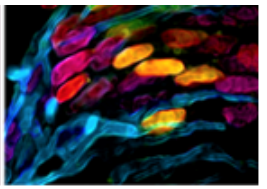
Neuroscience 2015

SHORT COURSE 3

Record Keeping and Data Management for High-Quality Science

Neuroscience 2016

SfN NEURONLINE Learning | Discussion | Neuroscience | VIRTUAL CONFERENCE



Mitigating Implicit Bias: Tools for the Neuroscientist

An SfN Virtual Conference | Jan. 23–24

Register Now

SfN's next virtual conference, Mitigating Implicit Bias: Tools for the Neuroscientist, begins next Wednesday, January 23, at 11 a.m. EST. SfN members can register now to gain complimentary access to the conference, in which experts will discuss topics such as negotiation and diversifying the workplace pipeline.

Hypothesis-driven research is based on scientific theories, while **exploratory** is based on a search for discovery backed by few theories or none at all (+ **data-driven** research)

NIH **guidelines:**

"A strong grant application is driven by a strong, solid hypothesis with clear research objectives. The specific aims are a formal statement of objectives and milestones of the research project towards testing the hypothesis."

- The research is driven by best practices (how to do and test science), and it's easy for peer reviewers to separate good from bad science based on the research methods
- Those with a realistic chance to prove what they set out to find and have the biggest impact for the public's benefit tend to receive the federal research dollars

Exploratory research, however, is driven more by hope and chance of discovery ("high-risk projects")

National Centre for the Replacement Refinement & Reduction of Animals in Research

Il disegno sperimentale

The survey revealed problems in the design, analysis and reporting of animal studies.

Only 12% of the 271 articles reported the use of **randomisation** and the use of **blinding** was reported in only 14% of the studies that used a qualitative outcome. This suggests that the vast majority of the studies made no attempt at reducing subjective bias that occurred when the animals were assigned to groups and when results were assessed.

Only 70% of the publications that used statistical methods fully described them and presented the results with a measure of precision or variability.

Only 59% of the studies included all three of the following important pieces of information: the hypothesis or objective of the study; the number of animals used; and characteristics of the animals (i.e.species/strain, sex, and age/weight).

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Il disegno sperimentale

Conducting a pilot study

A pilot, or feasibility study, is a small experiment designed to test logistics and gather information prior to a larger study, in order to improve the latter's quality and efficiency.

Example, for efficacy study:
-establish the end-points
-Information on precedure severity
-….

PRESENTAZIONE DI UN PROGETTO DI RICERCA AI SENSI DEL Art 31 D. L. 26/2014

17. **Razionale** dello studio
    - Stato delle conoscenze *(Giustificare lo studio con adeguati riferimenti bibliografici)*
    - Originalità e/o interesse dello studio *(valore scientifico)*
    - Eventuali ricadute nell'ambito della salute pubblica e/o animale *(valore sociale)*
    - Eventuale impatto nel settore economico-industriale *(valore economico)*
    - Eventuali ricadute nell'ambito della formazione *(valore didattico)*

18. Descrizione dei **fini** del progetto di ricerca (Art. 5, comma 1):
    - Ricerca di **base**
    - Ricerca **traslazionale** o applicata
    - Prove di tipo **regolatorio**

21. **METODOLOGIA E TECNICA** DELL'ESPERIMENTO *(va spiegato dettagliatamente …)*

21.1 Criteri di **selezione del campione** *(… criteri di inclusione e l'eventuale suddivisione dell'unità campionaria in gruppi)*

21.2 Considerazioni **statistiche** *(come è stato determinato il numero di animali)*

21.3 **Tecnica di esecuzione** della procedura *(descrivere il protocollo sperimentale con particolare riferimento alle procedure indicate in seguito)*

Che fare???

lab policy

- Clear study design (to be included in the methods section)
- Appropriate lab notebooks
- Materials concerns
- Statistical power: PoP vs efficacy vs safety studies
- Pre-registered statical analysis
- Blind + pre-registered exclusion criteria
- Biological vs technical replicates
- **Data repository**: Animal personal health record, Movies archives, Tissue banking, Cell banking, DNA banking

G*Power

Che fare???

Politiche istituzionali

RESEARCH COUNCILS UK *2009-2015*

RCUK Policy and Guidelines on Governance of Good Research Conduct

The concordat to support research integrity

201 Universities UK

21 MAY 2015 | VOL 521 | NATURE | 259

Publish or perish

Universities should release reports to show what they are doing to tackle misconduct — and funders should help them to do so effectively.

As we report on p… not bothering to do … very enlightening. … gated, and another c… claimed that the uni… year — an unlikely … takes the issue of mi…

"Pretending that research misconduct does not happen is no longer an option."

…at most universities are …e of the reports are not …umber of cases investi- …out a login. Four reports …zero investigations that …tensive university that …riously.

National Research Council of Italy

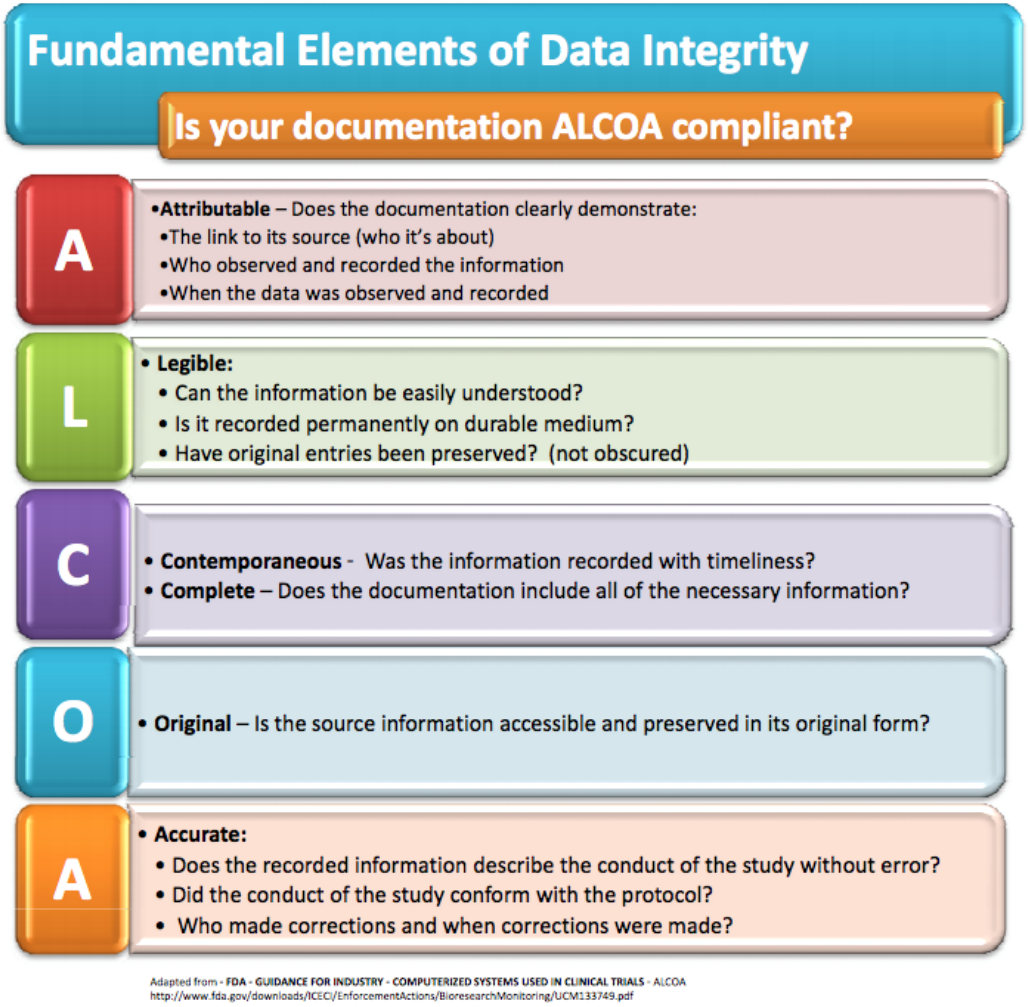
Università degli Studi di Napoli Federico II

Alma Mater Studiorum Università di Bologna

- Research Integrity guidelines(Good Science Practice)
- "condotta scorretta nella ricerca" (Research Misconduct)
- Regolamento di Ateneo per l'integrità scientifica nella ricerca
- Documento sull'integrità della ricerca

Possible initiatives

lab + editorial policies



- Focus on novelty
- Negative results
- Founding and Editorial policies *(errata corrige)*
- SOPs sharing
- Public raw data repository
- Replicates & Multicenter preclinical trials *(stroke, sci, …)*

Possible initiatives

scientific community policy

SPRINGER NATURE

Springer Nature Research Integrity Group (**SNRIG**)

Publication ethics & scholarly communication

A central resource for users to access Nature Research journals' policies on publishing ethics & scholarly communication: authorship, plagiarism, duplicate publication, competing interests, image integrity, confidentiality, preprints and conference proceedings.

Authorship

The Nature Research journals' authorship policy.

Find out more »

Duplicate publication

The Nature Research journals' policy on duplicate publication.

Find out more »

Plagiarism and fabrication

The Nature Research journals' policies on plagiarism and on providing due credit for published and unpublished data.

Find out more »

Image integrity

Nature Research journals' policies and guidelines on digital images and their manipulation.

Competing interests

Nature Research journals' compe policies.

Find out more »

Preprints and Conference Pr

Nature Research journals' policy and conference proceedings.

Find out more »

ELSEVIER

Research Integrity

Elsevier shares the research community's goal to promote the integrity of research – from proper design methodology to ethical article submission, properly reviewed publication, and making research data available for re-use. In the below we briefly list the many initiatives that we undertake to translate this vision into actual steps.

Science

AAAS

Science Translational Medicine

Study design:

Sample size
Rules for stopping data collection
Data inclusion/exclusion criteria
Outliers
Selection of endpoints

Replicates
Research objectives
Research subjects or units of investigation
Randomization
Blinding

Possible initiatives

scientific community policy

Cochrane Library

preclinical randomized controlled multicenter trial (pRCT)

"Meta-analysis is a quantitative, formal, epidemiological study design used to systematically assess previous research studies to derive conclusions about that body of research"

Meta-analysis

Multi-PART

Multicentre Preclinical Animal Research Team

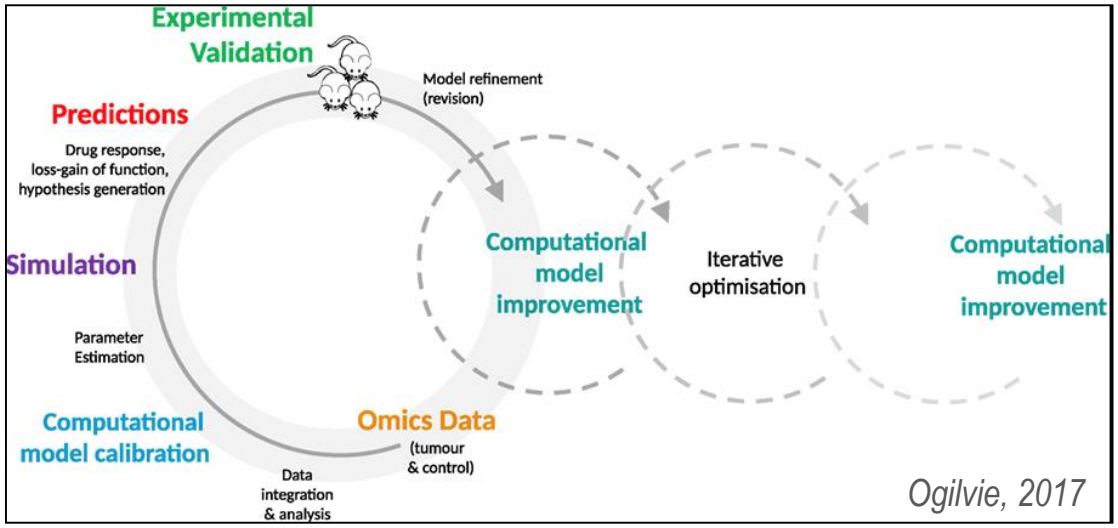
AAAS AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

pRCT: stroke mouse
a year and four months and cost a total of $180,000 or €165,000 (€30,000 for the cost of the drug)

5 August issue of Science Translational Medicine, 2015

Haidich, 2010

The next future



P4 Medicine

PREDICT PREVENT PERSONALIZE PARTICIPATE

In silico models

4P medicine

ML/AI

..... grazie .....

laura.calza@unibo.it
CIRI-SDV/Fabit
University of Bologna