Drug Studies Using Mouse Models

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NCI

Why should the taxpayers fund this type of work?

Cancer is the second leading cause of death in America. It accounts for one of every four deaths. More than 565,000 - that's more than 1,500 people a day - die annually from cancer. Close to 1.4 million new cases are diagnosed each year. This estimate does not include pre-invasive cancer or the more than 1 million cases of non-melanoma skin cancer expected to be diagnosed annually.

Why do we use mouse models?

To prevent, diagnose and treat human disease

- genetics
- progression
- prevention
- diagnostics
- therapeutics
- physiologically complex vs in vitro studies
- cost effective
- develop human clinical protocols

What models are available?

- Spontaneous
- Virus-induced
- Transgenic
- Knock-out/in
- Induced/carcinogens
- Transplanted

Spontaneous Models

These allow the study of the biological history of natural disease. They can be applied to many types of studies.

- Natural history is most "normal"
- Random tumors
- Difficult to predict occurrence
- Monitor through lifespan ~ 2 yr in mice
- Large number of animals may be required for a small experiment
- Tumors may be heterogeneous
- Significant animal holding space needed

Virus-Induced Models

Early studies of cancer, both as a disease state and for therapeutic intervention, used virus-induced models. These models continue to be applied in a variety of studies.

- Classic models of virus-induced leukemia:
  - Rauscher
  - Moloney
  - LP-BM5
  - Friend
- Non-leukemic tumors including:
  - Mammary tumors due to MMTV
  - Thymomas of AKR mice

- High tumor occurrence rate
- Predictable time to tumor development
- Well-characterized disease states/natural history
- Do not accurately reflect the natural history of most human tumors

Transgenic Models

Transgenic models aid our understanding of the genetics of cancer and are being pursued as models for intervention.

- Many available through commercial and collaborative arrangements
  - http://emice.nci.nih.gov/emice/mouse_models
  - http://mouse.ncifcrf.gov/
  - http://jaxmice.jax.org/query/f?p=205:1:1510299228434659165

- Not all patents have expired - check with OTT regarding legalities
- Remember MTAs are needed to receive or ship material that may have patent or licensing rights. This also applies to mice.

- Rate of tumor occurrence may be low
- Time to tumor occurrence may be difficult to predict
- Breeding schemes may be complex e.g., 3, 4 or more intermediate genetic crosses
- Genetic error(s) are generally well characterized
- Disease may follow a more natural course e.g., time to significant tumor burden may more accurately mimic the human; lesions likely relevant to their site of occurrence

Induced/Carcinogens

These models aid studies of the local, systemic, and environmental factors that influence tumor susceptibility, growth, and progression.

- Epithelial tumorigenesis
  - 7,12-dimethylbenz[a]anthracene [initiator mutagen] followed by multiple applications of 12-*O*-tetradecanoylphorbol-13-acetate [pro-inflammatory]
- GI tumorigenesis
  - 1,2 dimethlhydrazine-2-HCl
  - azoxymethane
- Sarcoma induction
  - Methylcholanthrene
- Lung tumorigenesis
  - nitrosamine 4- (methyl-nitrosamino)-1-(3-pyridyl)-1-butanone

Induced/Carcinogens

- Time to tumor development may be variable
- Mimics some human diseases very well
- Requires technical work with known carcinogenic agents thus special handling/equipment/facilities/training required
- Provides the entire natural history of tumor development to study

Transplanted Models

These models are commonly applied in studies of genetics, diagnostics, and medical interventions.

- Tissue Source
  - Syngeneic - same inbred strain – e.g., B16 tumors in C57Bl/6 mice
  - Allogeneic - same species, different strain (genetically diverse) – e.g., M5076 sarcomas in athymic mice
  - Xenogeneic - different species e.g., human, rat or dog tumors grown in immunocompromised mice
- Implant Site
  - Orthotopic
  - Heterotopic
- Endpoints

Transplanted Models

- Easy to control time of tumor occurrence
- Many tumor types/lines available
- Well accepted models
- Do not accurately recapitulate human disease
- Metastatic lesions are difficult to find
- Tumor growth rates may preclude multiple treatment cycles

Discovery & Development

production

In vitro studies

New therapy

efficacy trials

Pharmacology & toxicology

In Vivo Efficacy Models

- Human Tumors
  - Hollow fiber
  - Subcutaneous
  - Intravenous
  - Intraperitoneal
  - Orthotopic
    - Mammary fat pad
    - Intracranial
    - Intrarenal
    - Intrahepatic
    - Intracecal
- Rodent Tumors
  - Subcutaneous
  - Intravenous
  - Intraperitoneal
  - Orthotopic
  - Metastatic
  - Transgenic
  - Knock-in/out
  - Induced
  - Spontaneous

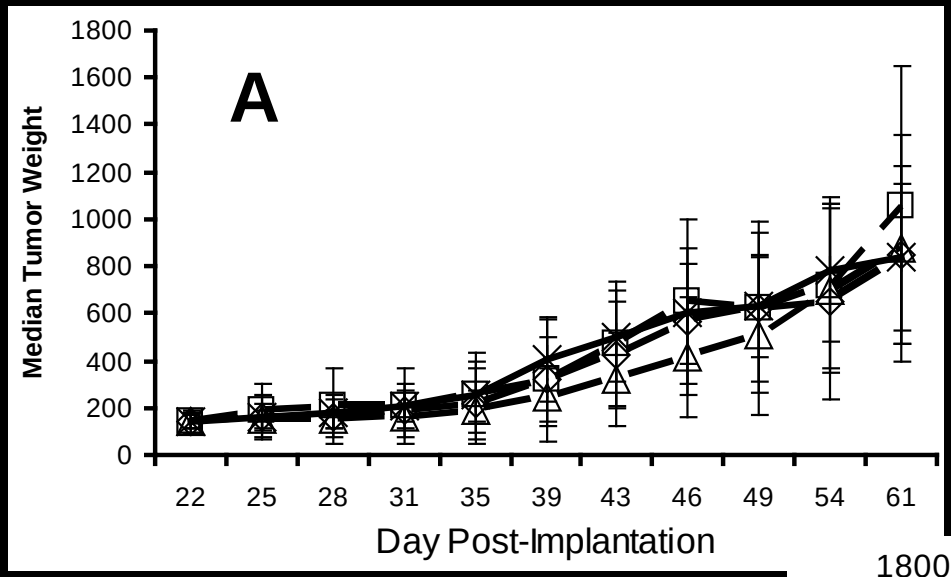
Issues faced during Efficacy Evaluations

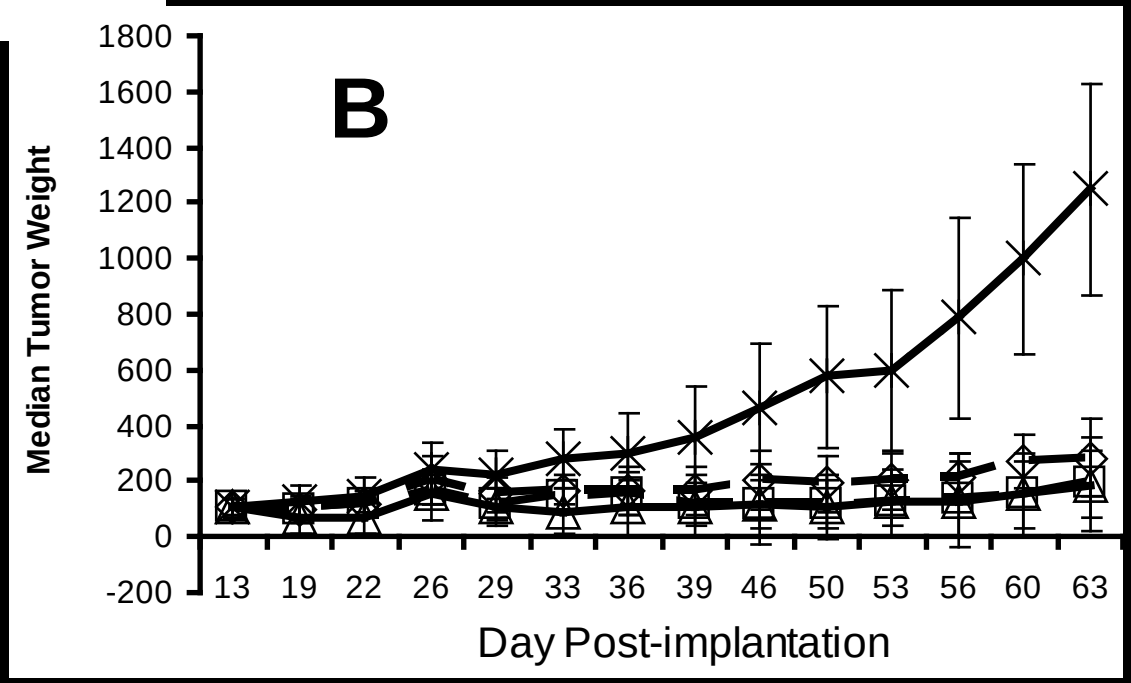
- Model
- Vehicle, formulation, stability
- Dose, route and schedule
- Experimental protocol
- Endpoints

Model Selection

- What are you assessing?
- Which type of model is most appropriate?
- Is the treatment designed to:
  - impact the tumor chemically, e.g., cytotoxic
  - impact the tumor genetically, e.g., modulator
  - impact the stroma e.g., vasculature
  - impact the immune system
  - act as an adjuvant
  - synergize with known drugs
  - interact with specific proteins

Model Selection





100% sesame oil

45 mg/kg tamoxifen

22.5 mg/kg tamoxifen

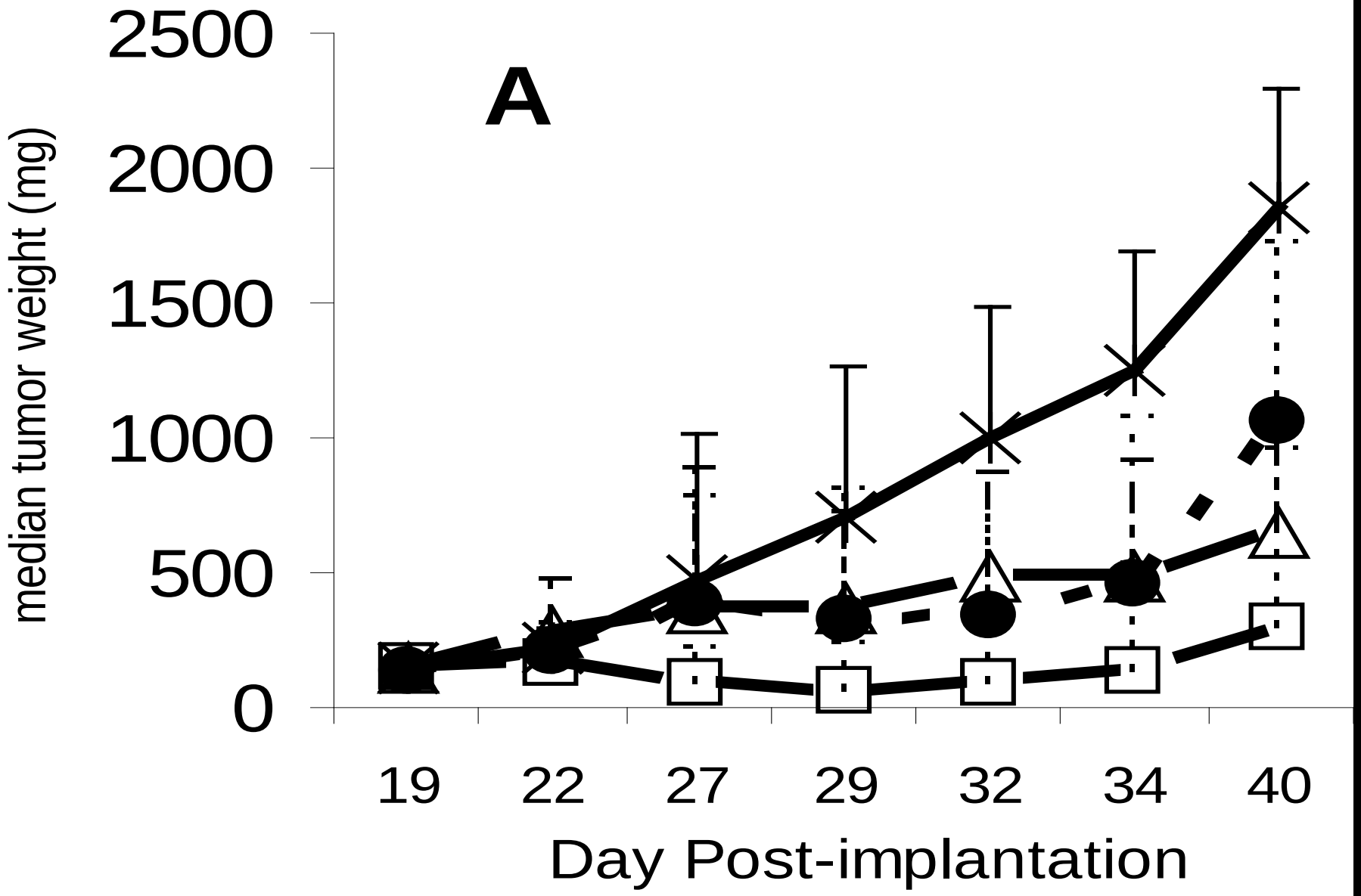
11.25 mg/kg tamoxifen

Experimental Protocol

- When will treatment start?
- When will treatment end?
- Will samples be collected for ex vivo evaluation?
- Will tumors be monitored visually, by imaging techniques?
- What will terminate the experiment, i.e., what are the endpoints?

Dose, Route, Schedule

- What is published/prior knowledge?
- What is proposed/expected mechanism?
- How much exposure is required for effect?
- Is the material soluble/stable in aqueous solution?
- What routes of administration are technically feasible?
- Options
  - What is the maximum tolerated dose (MTD)?
  - IP, IV, SC, PO?
  - QDx?; BIDx?; TIDx?



—×— vehicle

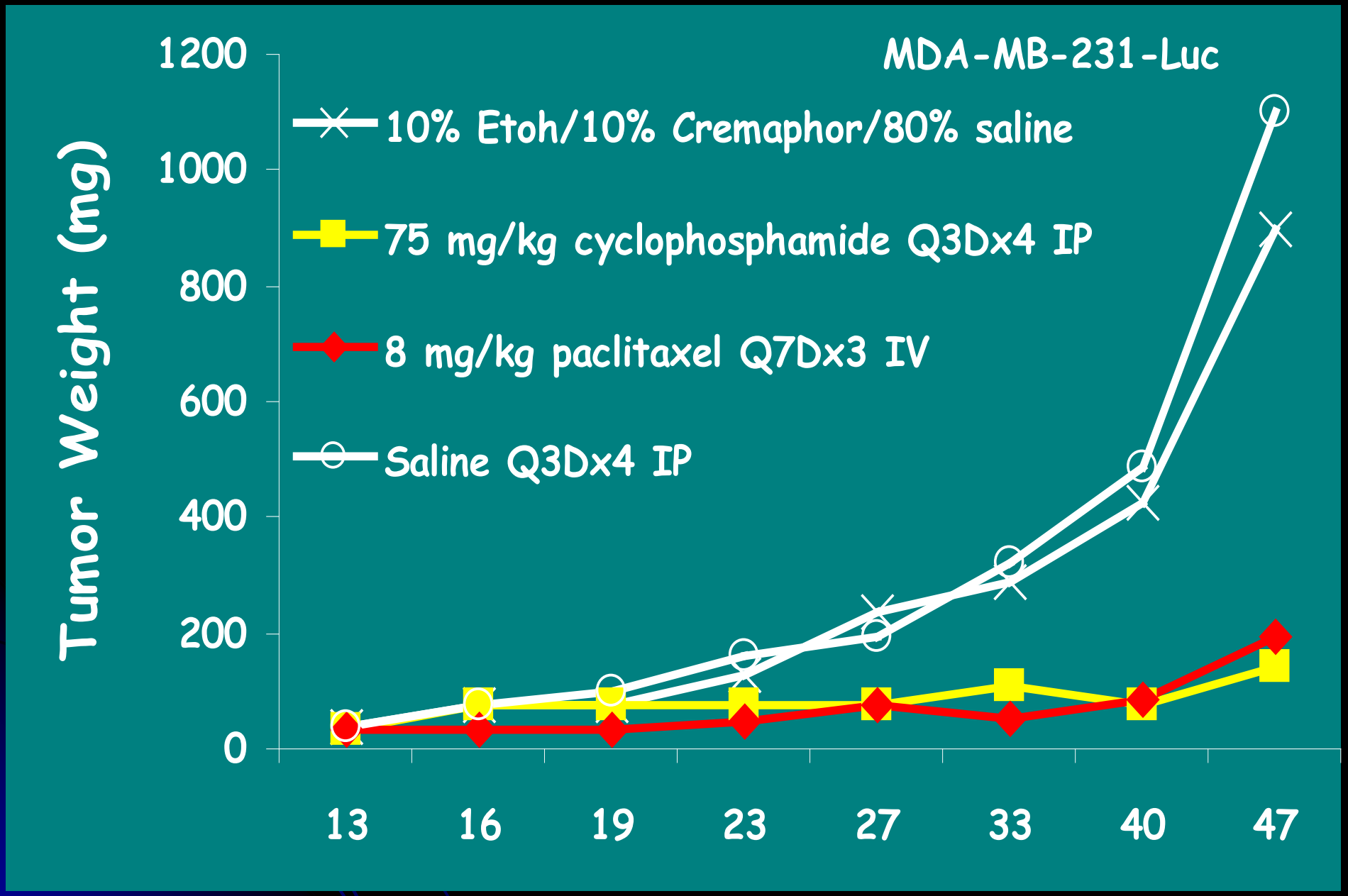
—Δ— temozolomide 200 mg/kg x 3

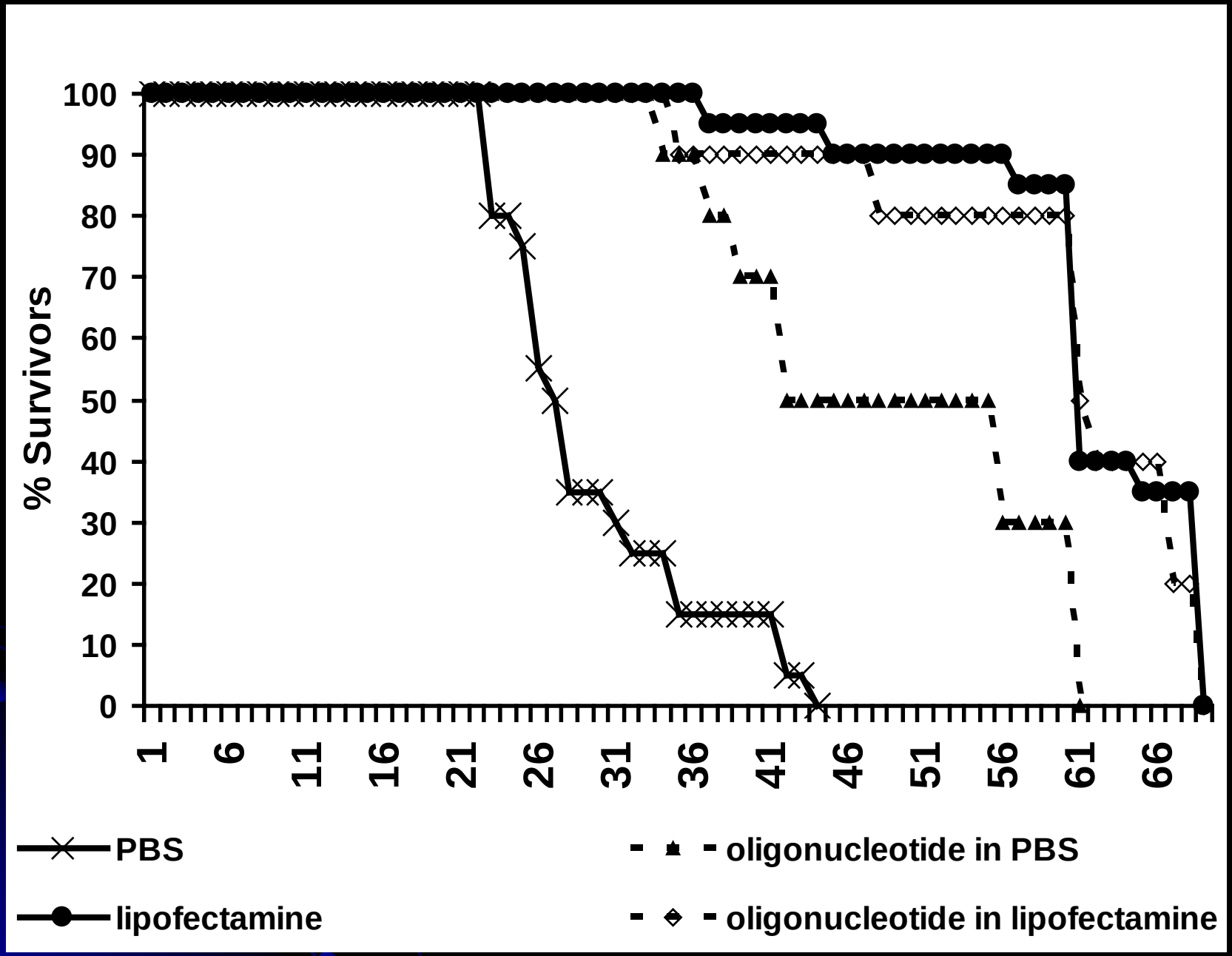
—□— temozolomide 400 mg/kg

—●— topotecan 1 mg/kg QDx10

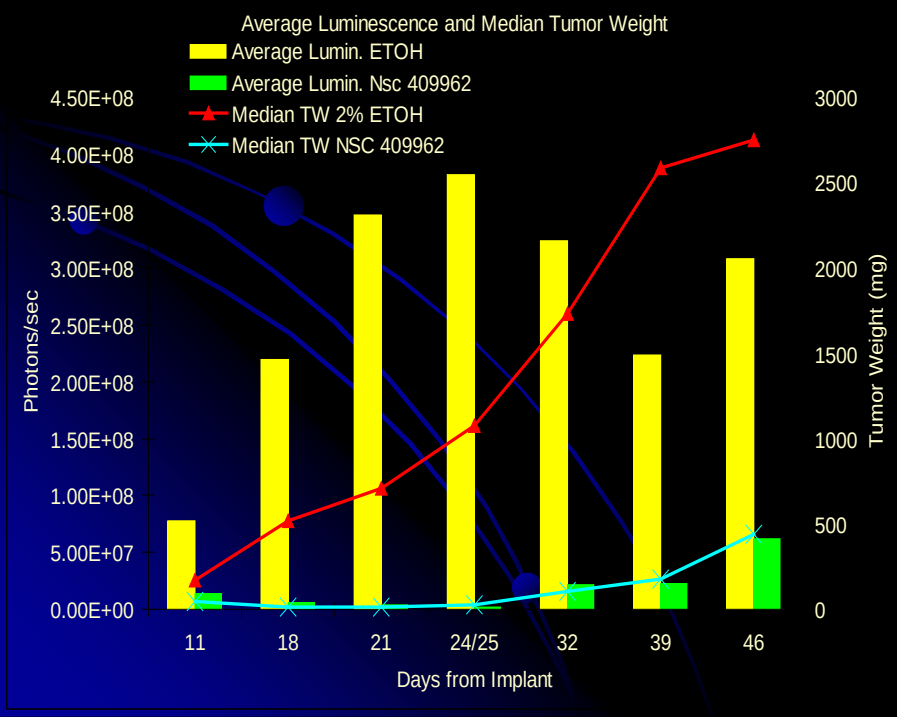
Endpoints

- Tumor size
- Weight loss
- Time to sacrifice
- Imaging
- Pre-defined time of termination
- Time post-treatment

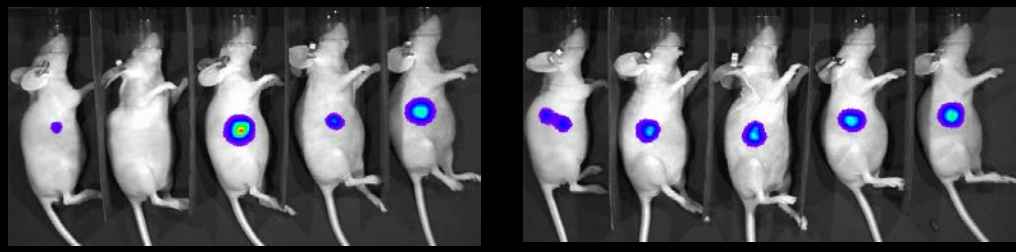

MDA-MB-231-Luc
10% Etoh/10% Cremaphor/80% saline
75 mg/kg cyclophosphamide Q3Dx4 IP
8 mg/kg paclitaxel Q7Dx3 IV
Saline Q3Dx4 IP
Tumor Weight (mg)
1200
1000
800
600
400
200
0
13
16
19
23
27
33
40
47


% Survivors
100
90
80
70
60
50
40
30
20
10
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
PBS
oligonucleotide in PBS
lipofectamine
oligonucleotide in lipofectamine

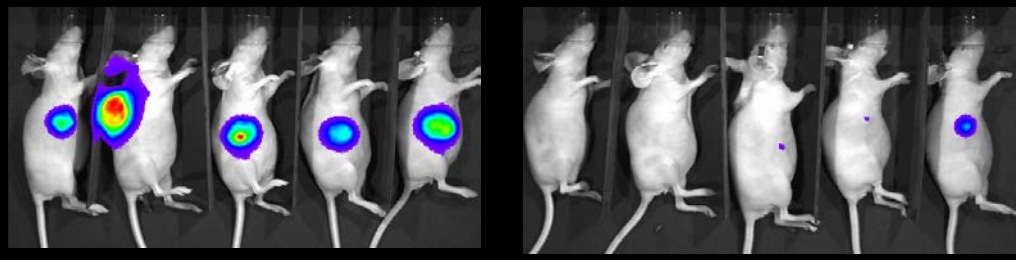
Measuring antitumor drug activity using bioluminescence vs. tumor weights



Vehicle BCNU

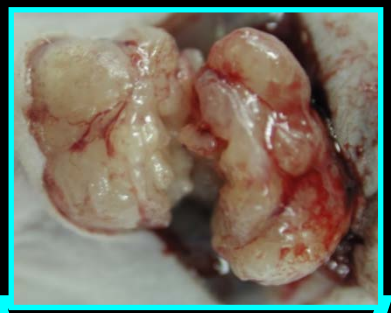


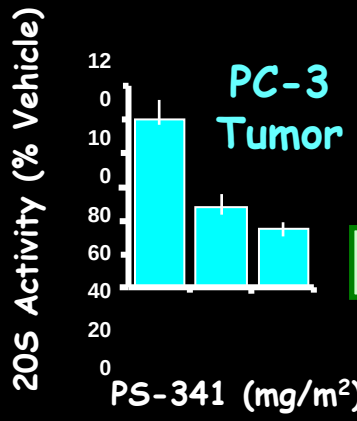
Prior to Treatment (Day 6)



7 days after third treatment (Day 21)

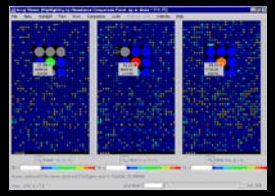
In Vivo Studies



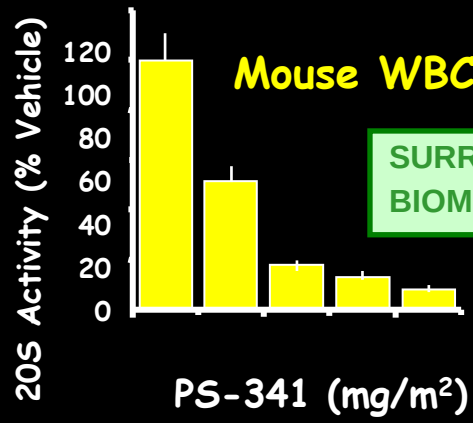


BIOMARKER

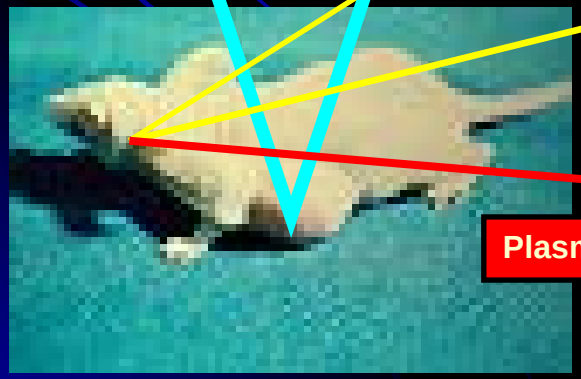
Tumor RNA



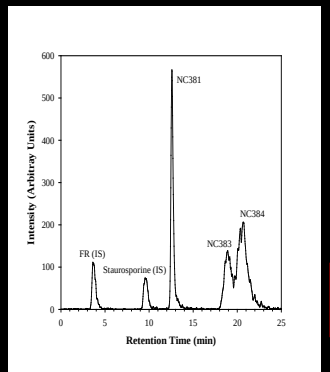
GENOMICS



SURROGATE BIOMARKER



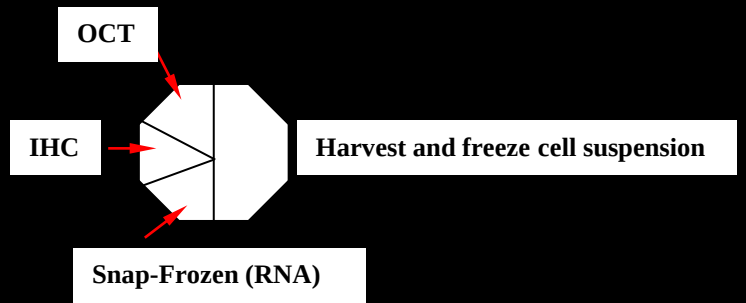
Plasma



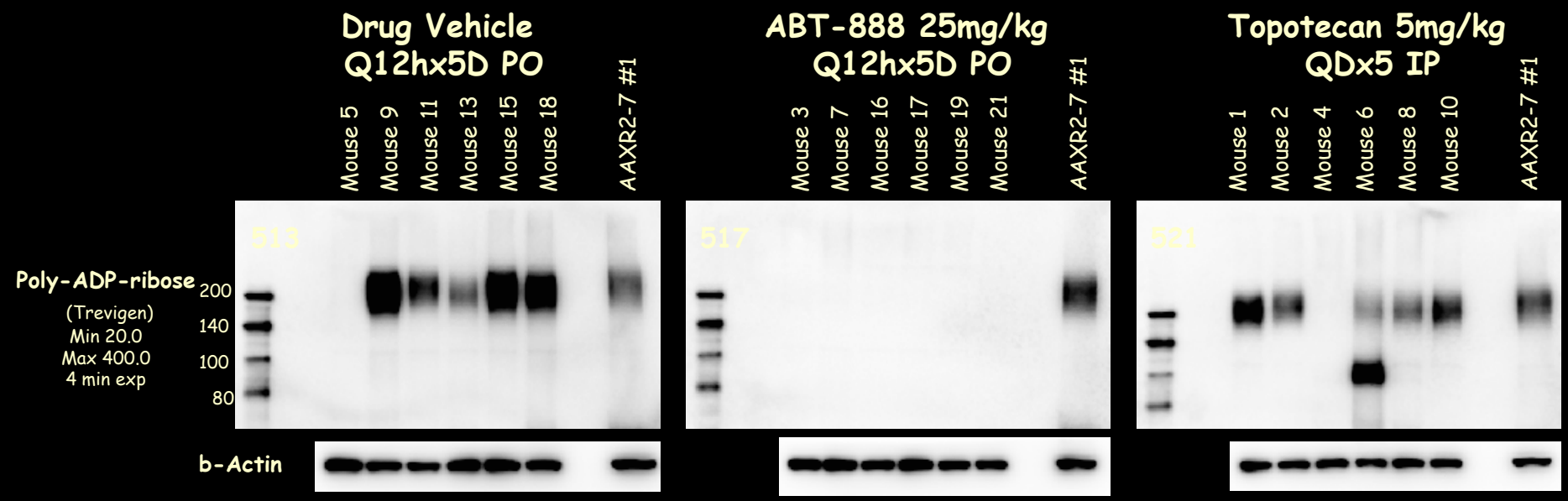
DRUG ANALYSIS

Tools for Analysis

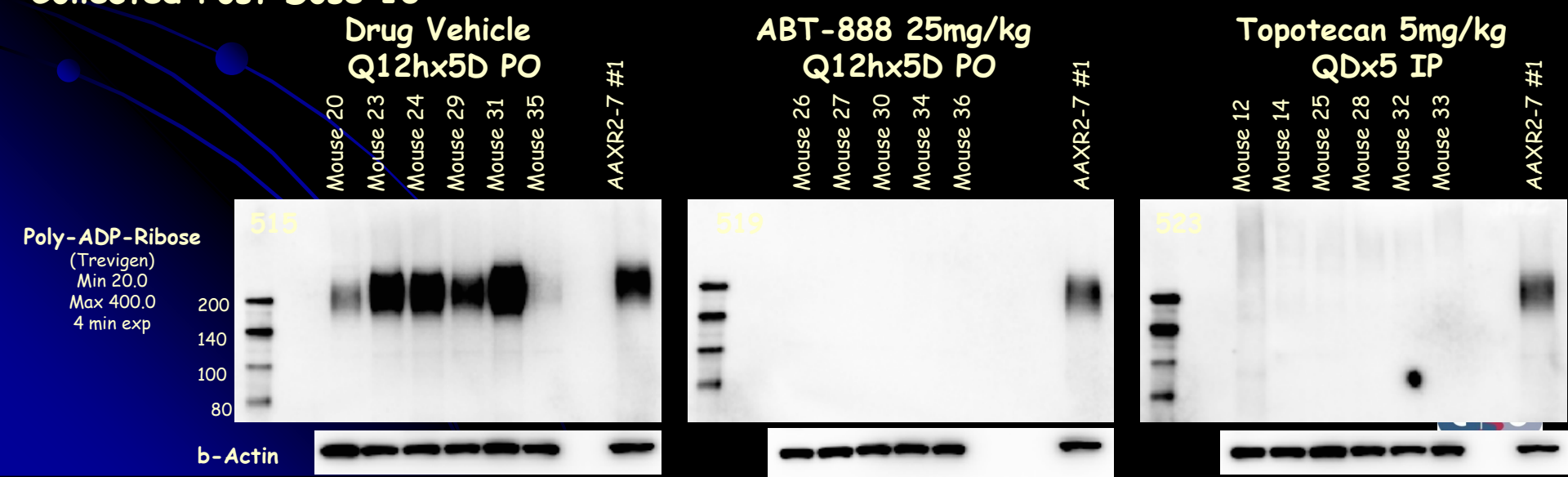
- IHC
  - Original tumor and each passage
  - Antibodies for specific gene products
- Molecular analysis
  - gene expression: microarray (LCM), RT-PCR
  - proteins/phosphoproteins: reverse arrays, IHC
  - biomarkers: serum proteomics
- Analysis of host response
  - immune system response (syngeneic v.s. immunosuppressed)
  - Angiogenesis (MFP v.s. sc)
- Stem Cell search



Colo-829 SC Tumors
Collected Post Dose 4



Colo-829 SC Tumors
Collected Post Dose 10



Many Models – Many Options

Statistically valid model assessing relevant endpoints on an optimal schedule with clinically appropriate doses.

With few exceptions, every rodent model, even if conducted with hundreds of experimental mice, represents a single patient. Interpreting the preclinical results and applying these outcomes to human clinical trials continues to prove challenging for those charged with translating the preclinical experience into viable drug candidates.

PROBLEMS

- The appropriateness of animal models to identify, qualify and promote new therapies for cancer has been under review, and in some ways under attack, for many years. Continuing concerns about the failure rate of agents being sent to the clinic has led to a flurry of publications on the irreproducibility of published preclinical data and their over-prediction of activity.

Opinionator

Exclusive Online Commentary From The Times

May 2, 2011, 9:15 PM

Helping New Drugs Out of Research's 'Valley of Death'

By DAVID BORNSTEIN

Fixes looks at solutions to social problems and why they work.

TAGS:

DRUGS (PHARMACEUTICALS), MEDICINE AND HEALTH, MULTIPLE SCLEROSIS, RESEARCH

Consider two numbers: 800,000 and 21.

The first is the number of medical research papers that were published in 2008. The second is the number of new drugs that were approved by the Food and Drug Administration last year.

That's an ocean of research producing treatments by the drop. Indeed, in recent decades, one of the most sobering realities in the field of biomedical research has been the fact that, despite significant increases in funding — as well as extraordinary advances in things like genomics, computerized molecular modeling, and drug screening and synthesization — the number of new treatments for illnesses that make it to market each year has flatlined (pdf) at historically low levels.

> An ocean of research is producing cures and treatments by the drop.

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How Many Scientists Fabricate and Falsify Research? A Systematic Review and Meta-Analysis of Survey Data

Daniele Fanelli*

INNOGEN and ISSTI-Institute for the Study of Science, Technology & Innovation, The University of Edinburgh, Edinburgh, United Kingdom

Abstract

The frequency with which scientists fabricate and falsify data, or commit other forms of scientific misconduct is a matter of controversy. Many surveys have asked scientists directly whether they have committed or know of a colleague who committed research misconduct, but their results appeared difficult to compare and synthesize. This is the first meta-analysis of these surveys. To standardize outcomes, the number of respondents who recalled at least one incident of misconduct was calculated for each question, and the analysis was limited to behaviours that distort scientific knowledge: fabrication, falsification, "cooking" of data, etc… Survey questions on plagiarism and other forms of professional misconduct were excluded. The final sample consisted of 21 surveys that were included in the systematic review, and 18 in the meta-analysis. A pooled weighted average of 1.97% (N = 7, 95%CI: 0.86–4.45) of scientists admitted to have fabricated, falsified or modified data or results at least once –a serious form of misconduct by any standard– and up to 33.7% admitted other questionable research practices. In surveys asking about the behaviour of colleagues, admission rates were 14.12% (N = 12, 95% CI: 9.91–19.72) for falsification, and up to 72% for other questionable research practices. Meta-regression showed that self reports surveys, surveys using the words "falsification" or "fabrication", and mailed surveys yielded lower percentages of misconduct. When these factors were controlled for, misconduct was reported more frequently by medical/pharmacological researchers than others. Considering that these surveys ask sensitive questions and have other limitations, it appears likely that this is a conservative estimate of the true prevalence of scientific misconduct.

Citation: Fanelli D (2009) How Many Scientists Fabricate and Falsify Research? A Systematic Review and Meta-Analysis of Survey Data. PLoS ONE 4(5): e5738. doi:10.1371/journal.pone.0005738

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Competing Interests: The author has declared that no competing interests exist.

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Do Pressures to Publish Increase Scientists' Bias? An Empirical Support from US States Data

Daniele Fanelli*

INNOGEN and Institute for the Study of Science, Technology and Innovation (ISSTI), The University of Edinburgh, Edinburgh, United Kingdom

Abstract

The growing competition and "publish or perish" culture in academia might conflict with the objectivity and integrity of research, because it forces scientists to produce "publishable" results at all costs. Papers are less likely to be published and to be cited if they report "negative" results (results that fail to support the tested hypothesis). Therefore, if publication pressures increase scientific bias, the frequency of "positive" results in the literature should be higher in the more competitive and "productive" academic environments. This study verified this hypothesis by measuring the frequency of positive results in a large random sample of papers with a corresponding author based in the US. Across all disciplines, papers were more likely to support a tested hypothesis if their corresponding authors were working in states that, according to NSF data, produced more academic papers per capita. The size of this effect increased when controlling for state's per capita R&D expenditure and for study characteristics that previous research showed to correlate with the frequency of positive results, including discipline and methodology. Although the confounding effect of institutions' prestige could not be excluded (researchers in the more productive universities could be the most clever and successful in their experiments), these results support the hypothesis that competitive academic environments increase not only scientists' productivity but also their bias. The same phenomenon might be observed in other countries where academic competition and pressures to publish are high.

Citation: Fanelli D (2010) Do Pressures to Publish Increase Scientists' Bias? An Empirical Support from US States Data. PLoS ONE 5(4): e10271. doi:10.1371/journal.pone.0010271

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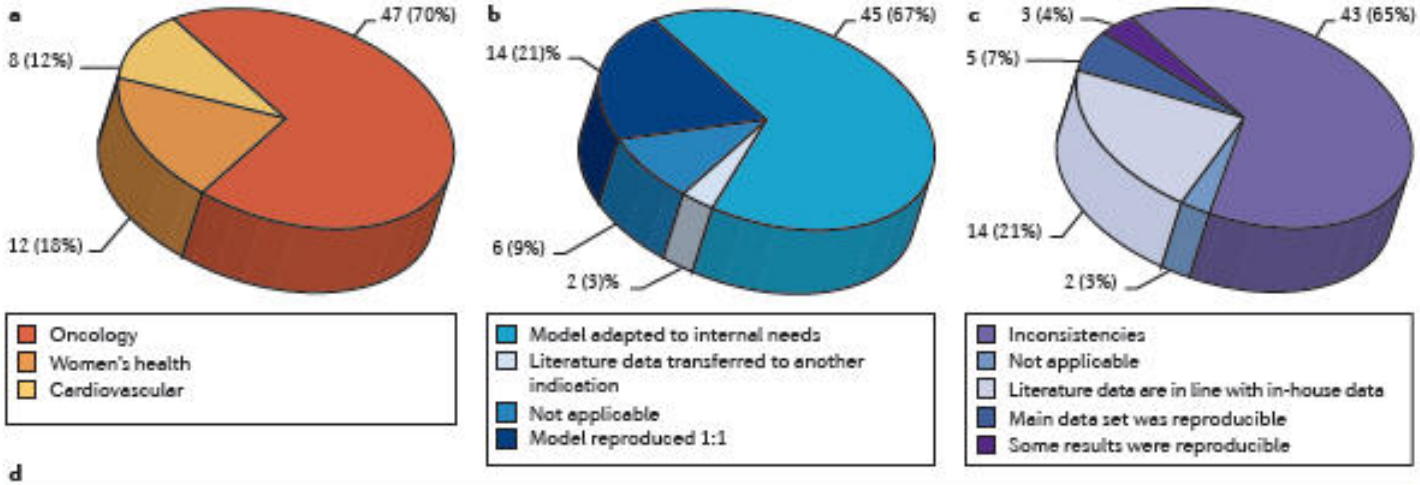
Believe it or not: how much can we rely on published data on potential drug targets?

Florian Prinz, Thomas Schlange and Khusru Asadullah

results that are published are hard to reproduce. However, there is an imbalance between this apparently widespread impression and its public recognition (for example, see REFS 2,3), and the surprisingly few scientific publications dealing with this topic. Indeed, to our knowledge, so far there has been no published in-depth, systematic analysis that compares reproduced results with published results for wet-lab experiments related to target identifica-



d

	Model reproduced 1:1	Model adapted to internal needs (cell line, assays)	Literature data transferred to another indication	Not applicable
In-house data in line with published results	1 (7%)	12 (86%)	0	1 (7%)
Inconsistencies that led to project termination	11 (26%)	26 (60%)	2 (5%)	4 (9%)

Figure 1 | **Analysis of the reproducibility of published data in 67 in-house projects. a** | This figure illustrates the distribution of projects within the oncology, women's health and cardiovascular indications that were analysed in this study. **b** | Several approaches were used to reproduce the published data. Models were either exactly copied, adapted to internal needs (for example, using other cell lines than those published, other assays and so on) or the published data was transferred to models for another indication. 'Not applicable' refers to projects in which general hypotheses could not be verified. **c** | Relationship of published data to in-house data. The proportion of each of the following outcomes is shown: data were completely in line with published data; the main set was reproducible; some results (including the most relevant hypothesis) were reproducible; or the data showed inconsistencies that led to project termination. 'Not applicable' refers to projects that were almost exclusively based on in-house data, such as gene expression analysis. The number of projects and the percentage of projects within this study (**a**–**c**) are indicated. **d** | A comparison of model usage in the reproducible and irreproducible projects is shown. The respective numbers of projects and the percentages of the groups are indicated.

CAVEAT!

Lab Mistakes Hobble Cancer Studies But Scientists Slow to Take Remedies

By Amy Dockser Marcus

Last year, cancer researcher Robert Mandic got news no scientist wants to hear.

After publishing a paper on a rare head-and-neck cancer, he learned the cells he had been studying were instead cervical cancer. He notified the journal Oral Oncology, which retracted the article.

"To base something on wrong data is bad, so it needs to be reported and I did," said Dr. Mandic, a researcher at the University Hospital Giessen and Marburg in Germany. "But it wasn't pleasant to call."

Dr. Mandic entered a largely secret fellowship of scientists whose work has been undermined by the contamination and misidentification of cancer cell lines used in research labs around the world.

Cancer experts seeking to solve the problem have found that a fifth to a third or more of cancer cell lines tested were mistakenly identified—with researchers unwittingly studying the wrong cancers, slowing progress toward new treatments and wasting precious time and money.

In hundreds of documented cases that undermine a broad swath of research, cancer samples that were supposed to be one type of tumor have turned out to be another, through either careless laboratory handling, mislabeling or other mistakes.

It is a problem hiding in plain sight. Warnings to properly test cancer cell lines have sounded since the 1960s, a decade after scientists started making human cancer cell lines.

But researchers who yelled loudest were mostly ignored by colleagues fearful such a mistake in their own labs would discredit years of work.

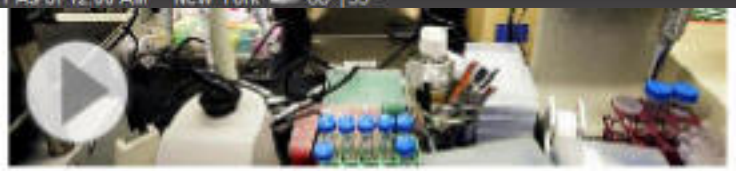
Leaders in the field say one of the biggest obstacles to finding a cancer cure may not be the many defenses nature affords malignancies, but the reluctance of scientists to address the problem.

"Screaming and shouting, it doesn't do any good. No one takes any notice for reasons I don't understand," said John Masters, a professor of experimental pathology at University College London, UCL. "The whole ethos of science is to strive for the truth and produce a balanced argument about the evidence. Yet, all this crap is being produced."

Dr. Masters said cell banks report that 20% of cell lines sent for inclusion in their repositories for use by researchers are improperly identified. He was co-chair of an international committee of scientists that released voluntary guidelines this year to begin solving the problem. They

Please turn to page A12

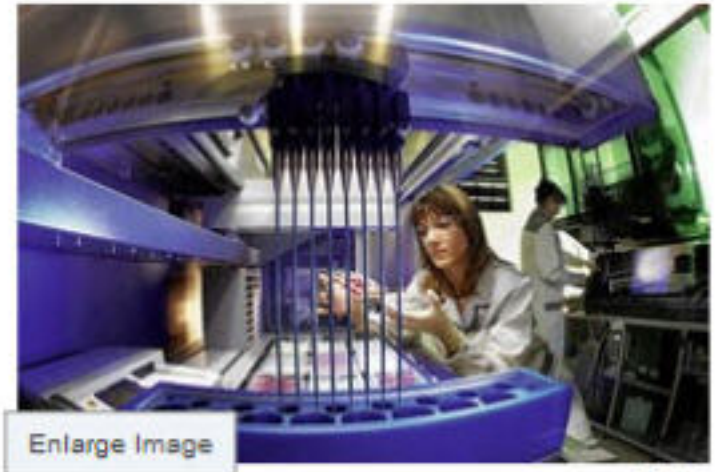
WSJ 4/24/12 Front Page



WSJ's Gautam Naik has details of challenges scientists face in reproducing claims made by medical journals. Photo: Sandy Huffaker/The New York Times

"I was disappointed but not surprised," says Glenn Begley, vice president of research at Amgen of Thousand Oaks, Calif. "More often than not, we are unable to reproduce findings" published by researchers in journals.

This is one of medicine's dirty secrets: Most results, including those that appear in top-flight peer-reviewed journals, can't be reproduced.



Enlarge Image

Bayer

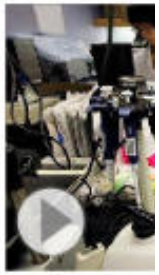
Researchers at Bayer's labs often find their experiments fail to match claims made in the scientific literature.

"It's a very serious and disturbing issue because it obviously misleads people" who implicitly trust findings published in a respected peer-reviewed journal, says Bruce Alberts, editor of Science. On Friday, the U.S. journal is devoting a large chunk of its Dec. 2 issue to the problem of scientific replication.

Reproducibility is the foundation of all modern research, the standard by which scientific claims are evaluated. In the U.S. alone, biomedical research is a $100-billion-year enterprise. So when published medical findings can't be validated by others, there are major consequences.

By GAUTAM N

Two years ag
had destroye
biotechnology
two dozen res
into a drug.



WSJ's Gautam Na

What can you do?

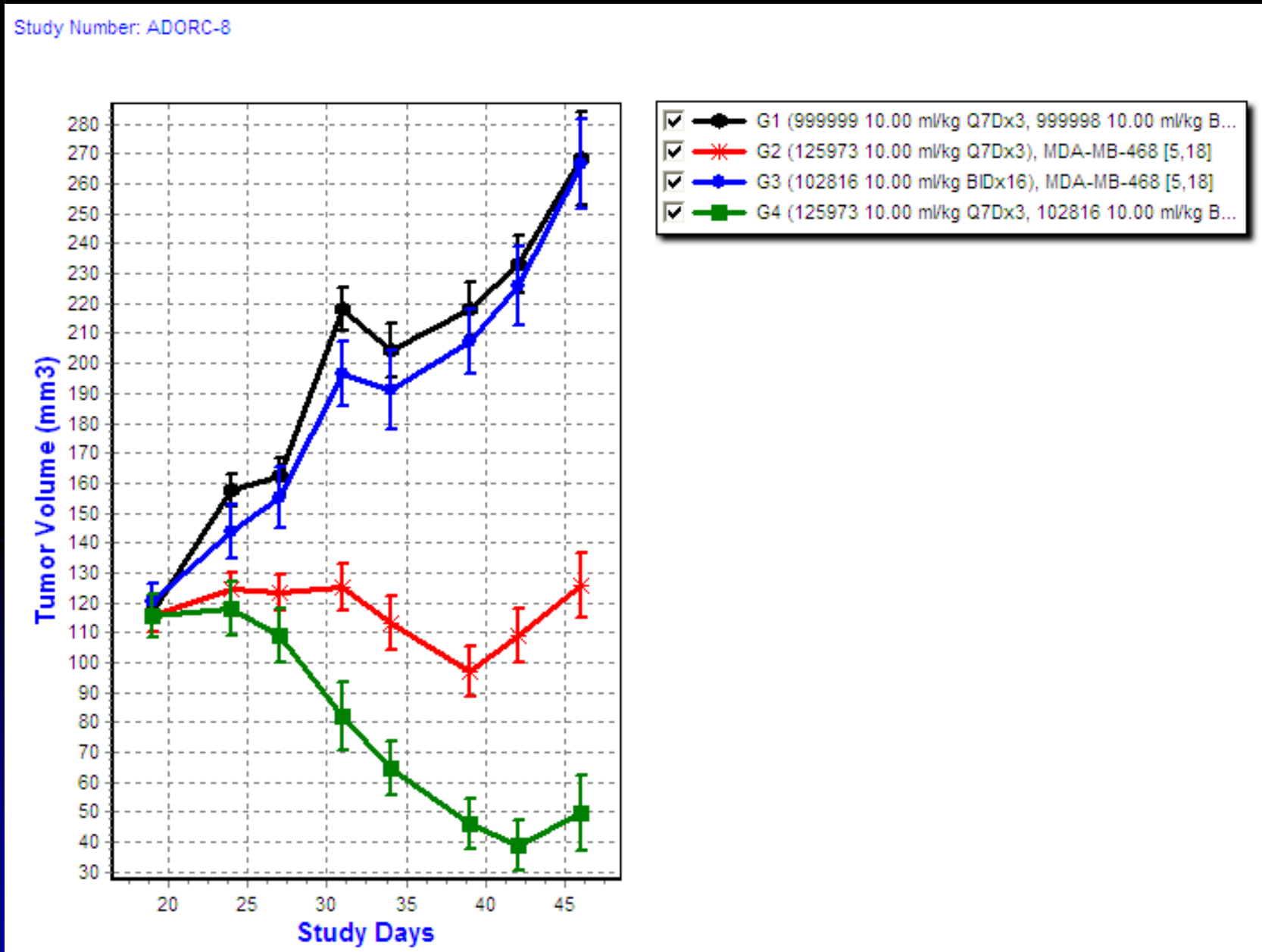
- 1. use well powered animal studies
- 2. reproduce your own data
- 3. have 2 separate operators generate the data
- 4. provide adequate details in publications
- 5. don’t over-interpret your data
- 6. stage tumor studies correctly
- 7. don’t selectively use/present your data
- 8. remember the clinical situation and what can be assessed in man

Clinical Endpoints In Man

- **Toxicity -** What harmful effects are induced?
- **Tumor response -** Does the cancer respond to the treatment?
  - Biomarker modulation as a measure of the effect of a treatment that may correlate with a traditional clinical endpoint (PFS; TR)
  - Progression-free survival (stable disease)
  - Tumor regression
  - Statistically significant improvement in survival

- **Survival -** how long does the person live?
- **Quality of life -** how does the treatment affect a person's overall enjoyment of life and sense of well being?

Progression, Stable, Regression



THANKS!!!

QUESTIONS???