Animals in cancer research
A. All five male BALB/c nu/nu mice in the XXXX group showed transplanted tumors after 28 days. B. Three mice of five in XXXX group showed small tumor after 28 days. C. The average tumor weight in XXXX group was significantly lighter than that in XXXX group (*P<0.05). D. The increase of xenografted tumor volumes in nude mice of the XXXX group was obviously slowed down comparing with the XXXX group (*P<0.05).

All animal experiments entirely obeyed the National Animal Care and Ethics Institution.
Journal Impact Factor: 3.96

### Reviewer #1

**Abstract needs to be rewritten so as to clearly summarize the main stuff of the study.**

**Response:** This is needed.

**Is it possible that the author could do some animal work to make sure in vivo?**

**Response:** This will be studied in the future.

**Make sure miR21 is also involved in CAS2/Caspase-7 regulative pancreatic tumor growth?**

**Response:** The in vivo studies will be studied in the future.

### Reviewer #2

**Title needs to be revised so as to clearly summarize the main stuff of the study.**

**Response:** This is needed.

**Some details about the authors need to be revised.**

**Response:** The author details are also listed in the list of authors.

**Make sure miR21 is also involved in CAS2/Caspase-7 regulative pancreatic tumor growth?**

**Response:** This is also involved in CAS2/Caspase-7 regulative pancreatic tumor growth.
It has become standard to use a mouse model in almost any cancer paper. (personal experience a journal with Impact Factor 2+ asked for mouse data)

The 3Rs only work when same rules for everyone
Review Article
Lost in translation: animal models and clinical trials in cancer treatment

Isabella WY Mak¹,², Nathan Evaniew¹,², Michelle Ghert¹,²

¹Department of Surgery, McMaster University, Hamilton, Ontario, Canada; ²Juravinski Cancer Centre, Hamilton Health Sciences, Hamilton, Ontario, Canada

Received December 20, 2013; Accepted December 5, 2013; Epub January 15, 2014; Published January 30, 2014

Indeed, animal studies seem to overestimate by about 30% the likelihood that a treatment will be effective because negative results are often unpublished [9]. Similarly, little more than a third of highly cited animal research is tested later in human trials [10]. Of the one-third that enter into clinical trials, as little as 8% of drugs pass Phase I successfully [11].
Point 1: Agree, negative data should be more accessible for the research community.

Point 2: Misleading.
Point 1: Agree, negative data should be more accessible for the research community.

Point 2: Misleading.

Point 3: Ten year-old non-peer reviewed strategy paper. How can 1 be true and 3? Paper ignores that cited strategy document actually offers improvement options.

So, am I saying there is no problem?
A tumour is considered to be lethal (depending on location and several other factors, such as hormonal secretion) if it reaches a weight of approximately 1 kg, or 10^{12} cells, although alternative numbers given suggest a maximum of 10^{13} cells.
Glioblastoma (GB) overview

- Most common and aggressive primary brain tumor in adults
- Diffuse and highly invasive growth pattern
- Median patient overall survival: 14.6 months
- Standard therapy consists of
  - Maximal safe surgical resection
  - Radiochemotherapy
  - Chemotherapy (temozolomide)
Proteins and Protein Pattern Differences between Glioma Cell Lines and Glioblastoma Multiforme

Timothy W. Vogel,1 Zhengping Zhuang,1 Jie Li,1 Hiroaki Okamoto,1 Makoto Furuta,1,2 Youn-Soo Lee,1,3 Wei Fen Zeng,1,4,5 Edward H. Oldfield,1 Alexander O. Vortmeyer,1 and Robert J. Wei1,6

Published OnlineFirst January 9, 2008; DOI:10.1158/1541-7786.MCR-07-0280

Genomic Changes and Gene Expression Profiles Reveal That Established Glioma Cell Lines Are Poorly Representative of Primary Human Gliomas

Aiguo Li,1 Jennifer Walling,1 Yuri Kotliarov,1 Angela Center,1 Mary Ellen Steed,1 Susie J. Ahn,1 Mark Rosenblum,2 Tom Mikkelsen,2 Jean Claude Zenklusen,1 and Howard A. Fine1

1Neuro-Oncology Branch, National Cancer Institute, National Institutes of Neurological Disorder and Stroke, NIH, Bethesda, Maryland and 2Neurology and Neurosurgery, Hemelin Brain Tumor Center, Henry Ford Hospital, Detroit, Michigan
Integrity of BBB: May Vary Within Tumor

- Ratio of tumor cells to total cells:
  - 1:1: 92%
  - 1:10: 6%
  - 1:100: 1.8%
  - 1:1000: 0.2%

- Percentage of tumor cell population:
  - 92%
  - 6%
  - 1.8%
  - 0.2%
The genomic profile of human malignant glioma is altered early in primary cell culture and preserved in spheroids
There are plenty of examples showing the benefits of using an animal model:

• For example, the breast cancer drug tamoxifen – arguably one of the most important cancer drugs of all time – was developed with the aid of animal research. Over the years, it has saved hundreds of thousands of women’s lives.
• The targeted drug imatinib (Glivec) can now cure people with chronic myeloid leukaemia. The original studies that identified imatinib’s potential were carried out in mice.
• The development of antibody treatments for cancer has also relied on animal research. Antibodies are molecules designed to recognise and target cancer cells, and early research in mice helped to find a way to produce large enough quantities of these molecules to be used to treat patients.
• Antibodies can now be made in industrial quantities without using animals, and these treatments are used for several types of cancer. New immunotherapy drugs called ‘checkpoint inhibitors’ which help the immune system recognise and attack cancer are just one example. These drugs have transformed the outlook for some people with advanced disease, such as melanoma, and wouldn’t have been possible without animal research.

https://scienceblog.cancerresearchuk.org/2011/06/21/animal-research-is-helping-us-beat-cancer/
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- Antibodies can now be made in industrial quantities without using animals, and these treatments are used for several types of cancer. New immunotherapy drugs called ‘checkpoint inhibitors’ which help the immune system recognise and attack cancer are just one example. These drugs have transformed the outlook for some people with advanced disease, such as melanoma, and wouldn’t have been possible without animal research.
Theralizumab
for the treatment of B cell chronic lymphocytic leukemia (B-CLL) and rheumatoid arthritis

In its first human clinical trials, it caused catastrophic systemic organ failures in the subjects, despite being administered at a supposed sub-clinical dose of 0.1 mg per kg; some 500 times lower than the dose found safe in animals. Six volunteers were hospitalized on 13 March 2006, at least four of these suffering from multiple organ dysfunction.

In 2010, the failure to predict a severe cytokine release syndrome in humans was explained with in vitro data of the CD4+ effector memory T-cells of Macaca fascicularis, the species of primate used for pre-clinical safety testing of TGN1412, lacking CD28 expression.

Average # of people joining a clinical trial

Fig. 3 Changes over time of clinical trials grouped according to age of subjects. Intervventional cancer-related clinical studies registered at ClinicalTrials.gov were categorized into four distinct groups, indicating whether the subjects were children, children and adolescents, adults or mixed (each study was allotted only one group). In a, absolute numbers are shown, thus indicating the development of the number of oncology studies over time, while in b, the distribution of the target age group of the oncology studies over time (in % of total) is depicted. Of note, the time period 1993–1998 precedes the establishment of the database and therefore only contains few appended entries, and studies without identifying starting date or with future starting date were excluded (cutoff date: 22 May 2017).
Too many targets, not enough patients: rethinking neuroblastoma clinical trials

Jamie I. Fletcher, David S. Ziegler, Toby N. Trahair, Glenn M. Marshall, Michelle Haber and Murray D. Norris

Table 1: Recurrent aberrations in neuroblastoma at diagnosis and relapse

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Diagnosis frequency (%) (n = 240)</th>
<th>Diagnosis frequency (%) (n = 230)</th>
<th>Relapse frequency (%) (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK mutation</td>
<td>9.2</td>
<td>14.3</td>
<td>24.7</td>
</tr>
<tr>
<td>PTPN11 mutation</td>
<td>2.9</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td>ATRX mutation</td>
<td>2.5</td>
<td>1.8</td>
<td>11.1</td>
</tr>
<tr>
<td>ATRX deletion</td>
<td>7.1</td>
<td>4.0</td>
<td>5.6</td>
</tr>
<tr>
<td>MYCN mutation</td>
<td>1.7</td>
<td>0.9</td>
<td>3.7</td>
</tr>
<tr>
<td>MYCN amplification</td>
<td>32.0</td>
<td>25.7</td>
<td>18.5</td>
</tr>
<tr>
<td>NRAS mutation</td>
<td>0.8</td>
<td>2.6</td>
<td>7.4</td>
</tr>
<tr>
<td>NF1 mutation or truncation</td>
<td>0</td>
<td>2.2</td>
<td>5.6</td>
</tr>
<tr>
<td>KRAS mutation</td>
<td>0</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>FGFR1 mutation</td>
<td>0</td>
<td>1.7</td>
<td>9.3</td>
</tr>
<tr>
<td>TP53 mutation</td>
<td>0.8</td>
<td>3.5</td>
<td>74</td>
</tr>
</tbody>
</table>

*Includes variants of unknown significance.

10.3 per 1M children
Neuroblastoma a copy number disease
Relapse a mutational disease, more drugable targets
In the traditional 3 + 3, phase I cancer trial design, a minimum of three participants are studied at each dose level. If none of these three participants experience a DLT (dose limiting toxicity), a subsequent three participants are enrolled onto the next highest dose level.

The rolling six design allows for accrual of two to six patients concurrently onto a dose level. Decisions as to which dose level to enroll a patient are based on the number of patients currently enrolled and evaluable, the number of patients experiencing DLTs, and the number of patients still at risk of developing a DLT at the time of new patient entry.
Pharmacokinetic studies redesigned

Traditional PK study design

In a traditional study each group of animals receives a single dose of the medicine, and groups are compared to each other at the end of the study. In a crossover design, each animal receives multiple doses of the drug over a period of several weeks or months. This allows multiple data sets to be collected from a single animal, and reduces variability in the data by allowing the effects of the medicine to be compared to the effects of no medicine within a single animal. This reduces the total number of animals needed in the study (see diagram to right).

Crossover design

<table>
<thead>
<tr>
<th>WEEK 1</th>
<th>WEEK 2</th>
<th>WEEK 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>Vehicle*</td>
<td>Low Dose</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>Low Dose</td>
<td>Vehicle*</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>High Dose</td>
<td>Vehicle*</td>
</tr>
</tbody>
</table>

Compare between groups

Better science (less variation) and fewer animals

*Vehicle is the solution in which the drug is dissolved. It may be water or another solvent which has minimal effects on the animal. One group is given the vehicle alone, without drug, as a control group.
Intravital Imaging Reveals How BRAF Inhibition Generates Drug-Tolerant Microenvironments with High Integrin β1/FAK Signaling

Authors
Eihu Hirata, Maria Romina Girotti, ..., Richard Marais, Erik Sahai

Correspondence
erik.sahai@crick.ac.uk

In Brief
Hirata et al. show that the BRAF inhibitor PLX4720 promotes melanoma-associated fibroblasts in BRAF-mutant melanomas to produce and remodel matrix, leading to integrin β1-FAK-Src signaling and reactivation of ERK and MAPK in melanoma cells. Co-inhibition of BRAF and FAK blocks ERK reactivation.

Highlights
- BRAF mutant melanoma cells respond to PLX4720 heterogeneously in vivo
- BRAF inhibition activates MAFs, leading to FAK-dependent melanoma survival signaling
- ECM-derived signals can support residual disease
- BRAF and FAK inhibition synergize in pre-clinical models
A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response

Zhao Chen1,2,3, Katherine Cheng2,3, Zandra Walton2,3, Yuchuan Wang4,5,6, Hiromichi Ebi1,7, Takeshi Shimamura8, Yan Liu1,2,3, Tanya Tupper4, Jing Ouyang2, Jie Li9, Peng Gao2,3, Michele S. Woo2, Chunxiao Xu1,2,3, Masahiko Yanagita2, Abigail Altabel2, Shumei Wang10, Charles Lee10, Yuji Nakada11, Christopher G. Peña1,11, Yanping Sun15, Yoko Franchetti12, Catherine Yao2, Amy Saur1, Michael D. Cameron13, Mizuki Nishino5,6, D. Neil Hayes14, Matthew D. Wilkinson14, Patrick J. Roberts14, Carrie B. Lee14, Nabeel Bardeesy7, Mohit Butaney2, Lucian R. Chiriac10, Daniel B. Costa15, David Jackman2, Norman E. Sharpless14, Diego H. Castrillon11, George D. Demetri3, Pasi A. Jänne1,2,16, Pier Paolo Pandolfi17, Lewis C. Cantley18,19, Andrew L. Kung4,20, Jeffrey A. Engelman1,7, and Kwok-Kin Wong1,2,3,16

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Genotype</th>
<th>Partial response (% &gt;30% regression)</th>
<th>Stable disease %</th>
<th>Disease progression % (&gt;30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel</td>
<td>Kras</td>
<td>30</td>
<td>55</td>
<td>15</td>
</tr>
<tr>
<td>Selumetinib +</td>
<td>Kras</td>
<td>92</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>docetaxel</td>
<td>Kras/p53</td>
<td>5</td>
<td>28</td>
<td>67</td>
</tr>
<tr>
<td>Selumetinib +</td>
<td>Kras/p53</td>
<td>61</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>docetaxel</td>
<td>Kras/Lkb1</td>
<td>33</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>Selumetinib +</td>
<td>Kras/Lkb1</td>
<td>33</td>
<td>50</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mutation status</th>
<th>Number of patients</th>
<th>Average pERK score</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>33</td>
<td>0.74</td>
</tr>
<tr>
<td>LKB1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>p53</td>
<td>15</td>
<td>0.4</td>
</tr>
<tr>
<td>LKB1/p53</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>KRAS</td>
<td>2</td>
<td>0.75</td>
</tr>
<tr>
<td>KRAS/LKB1</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>KRAS/p53</td>
<td>3</td>
<td>2.2</td>
</tr>
</tbody>
</table>
LMO1 Synergizes with MYCN to Promote Neuroblastoma Initiation and Metastasis


1Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Mayo Clinic Cancer Center, Rochester, MN 55902, USA
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8Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
9Department of Dermatology, Mayo Clinic, Rochester, MN 55902, USA
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11Abramson Family Cancer Research Institute, Philadelphia, PA 19104, USA

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http://dx.doi.org/10.1016/j.cell.2017.08.002
Drosophila melanogaster (fruit or vinegar fly)

• It’s been assumed that Drosophila doesn’t get cancer (short lived organism), but...

• D. melanogaster tumours range from hyperplasias to frankly malignant neoplasias that are invasive and lethal to the host.

• The Aurora and POLO protein kinases are tumour suppressors in the larval brain.

• Not an animal experiment

Social environment mediates cancer progression in *Drosophila*

Erika H. Dawson, Tiphaine P. M. Bailly, Julie Dos Santos, Céline Moreno, Maëlle Devilliers, Brigitte Maroni, Cédric Sueur, Andreu Casalí, Beata Ujvari, Frederic Thomas, Jacques Montagne & Frederic Mery

The influence of oncogenic phenomena on the ecology and evolution of animal species is becoming an important research topic. Similar to host-pathogen interactions, cancer negatively affects host fitness, which should lead to the selection of host control mechanisms, including behavioral traits that best minimize the proliferation of malignant cells. Social behavior is suggested to influence tumor progression. While the ecological benefits of sociality in gregarious species are widely acknowledged, only limited data are available on the role of the social environment on cancer progression. Here, we exposed adult *Drosophila*, with colorectal-like tumors, to different social environments. We show how subtle variations in social structure have dramatic effects on the progression of tumor growth. Finally, we reveal that flies can discriminate between individuals at different stages of tumor development and selectively choose their social environment accordingly. Our study demonstrates the reciprocal links between cancer and social interactions and how sociality may impact health and fitness in animals and its potential implications for disease ecology.
• heat shock (HS)-induced MARCM (Mosaic analysis with a repressible cell marker) clones were created in 3-day old adult females, knocking out both copies of APC and expressing oncogenic RASv12
Alone: one cancerous virgin fly alone for 21 days
Homogeneous: one cancerous virgin fly kept with seven other cancerous flies
Heterogenous: one cancerous virgin fly kept with seven other healthy flies

Letters: same letter means no significant difference between groups, here a is significantly different from the two b’s
This is not a good way to do it, plus significance is nowhere defined, assume: p<0.05?

Fig. 1 Gut tumor progression as a function of social environment. FACS analysis of GFP-positive cells in guts dissected from 21 days post-HS cancerous females as a function of social environment. Blue dots indicate mean value for each replicate. Error bars: standard error of the mean. N = 15 measures for each treatment. Letters are Tukey’s post-hoc classification.
Conclusions:

• There need to be stringent rules by the publishing community regarding animal data (and not just lip service)

• I am a big fan of self-regulation in the scientific community, Asilomar Conference 1975 (but I originally wrote this on the day the first CRISPR/Cas twins were announced, so what do I know)

• Animal experiments are expensive and time consuming, we all like acceptable alternatives – problem is how to make alternatives acceptable

• There are many examples in cancer research were animal research has been essential. After all, the law states drugs must be tested in animals first.