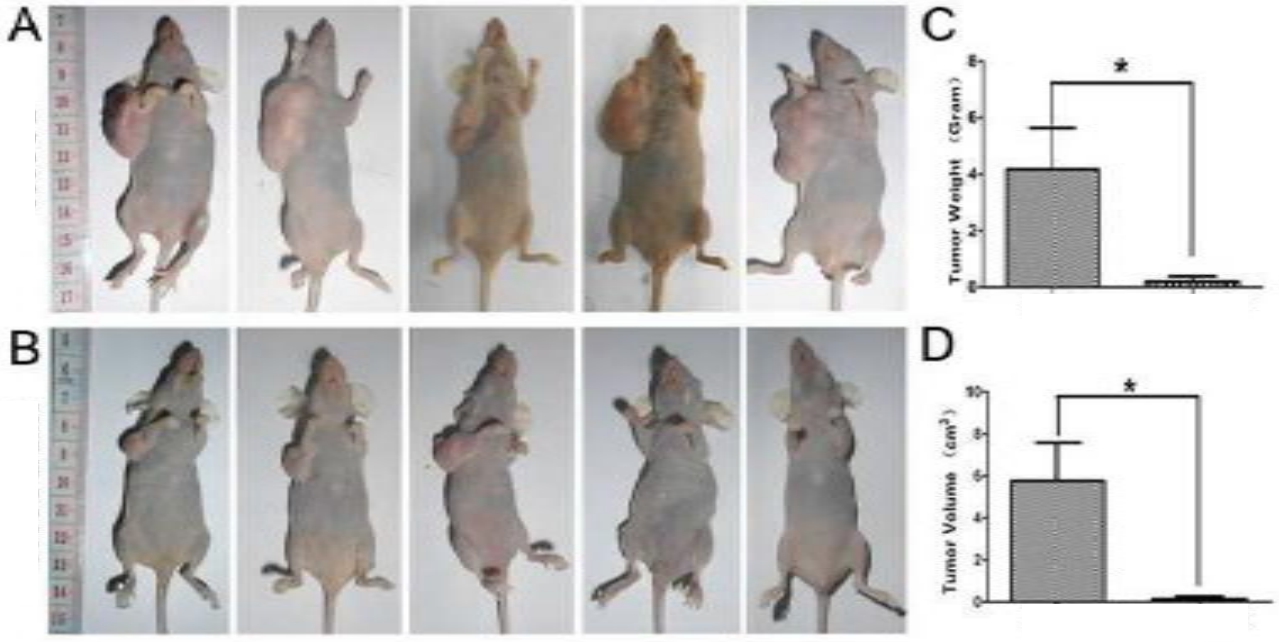
Animals in cancer research

Journal Impact Factor: 3.96



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 reports:
Reviewer #1:
1. Metastasis is the establishing of a secondary malignancy at a distant site, distinct from the tissue of origin. Establishing metastases is a complex process involving many distinct aspects, such as EMT, loss of cell-cell interactions, motility, directed movement, invasion, survival in hostile microenvironment etc. This paper looks at directed motility and invasion through a ECM-like matrix (although see point 2), this is not metastasis, but just two aspects that a involved in metastasis. This needs to be made clearer in the title and main text.
Response: It is revised to "migration and invasion" now.

2. 48 hrs is a very long time for a transwell assay, the gradient created by 10% FCS addition is no longer measurable within 24 hrs. In addition, any experiment lasting longer than 24 must take proliferation into account, especially when dealing with molecules which have been already characterised as being involved in proliferation (acknowledged in manuscript). It is possible that perceived differences in directed motility and invasion are the result of differences in proliferation. The proliferation rate of pancreatic cancer cell lines is being debated, but Panc-1 has been described as having a doubling time of 28 hrs (while originally 62 hrs where suggested). Cell lines have been characterised, for example, by Deer et al, 2010 in Pancreas - a reference which should be included in the manuscript (disclosure: not the author of this publication, not affiliated with any one who is the author and as far as I know never met any of them).
The authors need to show that after 48 hrs the same about of cells is present in the populations compared (i.e. no differences in growth rate or spontaneous apoptosis have occurred to explain differences in invasion/directed motility).
Response: The reference is cited now. It's very important to consider the proliferation of cells that might influent the migration results. However, the proliferation of the migrated cells could be disregarded at 24 h. We removed the cells in the upper surface of the membrane, and then co-continued to culture the cells on the lower surface for 24 h. No significant increases in cell number were observed. In this manuscript, we make a description in the Material and methods section now.

3. 1.25 pages long discussion spends 0.25 pages discussion the findings of Yu et al., 2017 (reference 9) and another 0.25 pages restating information given in the Introduction. Putting the results into context, discussion the limitations as well as the relevance of their own findings should be the main focus of this section.
Response: The discussion is revised.

Minor concerns:
1. Abstract:
"The mechanism of pancreatic cancer metastasis remains unclear." - that's not true, a lot is known about the underlying molecular mechanisms of metastasis!
"CASC2 exhibiting as a tumour suppressor in pancreatic cancer cells to" -functions not exhibiting
Response: These are revised.

2. Introduction:
"In recent years, its incidence has been increasing year by year" - annually is better than year by year, this and several other statements lack referencing
"In recent years, with the development of molecular biology, the study of tumors has been deepened to the level of genes" - hardly in recent years, first Oncogenes were discovered in the 1970s
"cancer-specific genes" - most genes are not cancer-specific, their expression might be, the mutations found might be, but cancer-specific genes like Bcr-Abl are rare.
lncRNA/mRNA/miRNA etc are spelled in different ways throughout the manuscript, sometimes with a space sometimes without, sometimes capitalised sometimes not.
"Cancer susceptibility candidate 2 (CASC2) is a recently discovered" - 2004 not recent
"metastasis of glioma cells" - Glioma very rarely metastasizes (~1.2% of all cases), it is however highly invasive- which is my point in the major concerns section.
"Metastasis and other related genes" - metastasis is not a gene, the sentence is unclear.
"In addition, resveratrol could induce apoptosis of pancreatic cancer cells by inhibiting miR-21
[21]." - relevance of this sentence should be expanded on.
Response: These are revised.
Unclear why the authors didn't also look at miR-193/367/181a.
Response: This study is focused on miR-21.
3. Material and methods
The section on cell migration and invasion is very brief.
4. Results
"CASC2 is low expressed in" - Expression of CASC2 is low
"including CAPAN-1, BxPC-3, JF305, PANC-1 and SW1990 and human normal pancreatic HPDE6-C7" - technically it is not including if all members of a group are mentioned
"In addition, cotransfection of miR-21 mimics and CASC2-wt significantly decreased the luciferase activity (P<0.001, Figure 2C), but cotransfection of miR-21 and CASC2-mut not changed the luciferase activity. These results suggested that miR-21 was a direct target of CASC2. MiR-21 mimics significantly increased the miR-21 levels in pEX CASC2 transfected PANC-1 cells (P<0.01, Figure 2D)." - this bit is suddenly very technical and cannot be understood without reading the Material and methods section, revising it would help the reader.
Response: These are revised.
5. Discussion
"The overexpression of miR-21 by miR-21 significantly" - something went wrong here.
Response: This is revised.
6. Conclusion
"via sponging miR-21." - that wasn't shown in this paper!
Response: These are revised.
7. Figures
Fig 2A why only look at two cell lines? Lysates should be there from Fig 1A
Response: The lysates were performed.
Fig 3 B versus C, the relative expression of PTEN in CT and pEX CASC2 (pEX CASC2+mimics NC) differs between B and C more than two-fold. That does not inspire confidence in the reliability of the densiometric analysis and might be worth commenting on.
Response: The protein loading concentration is different for Fig.3B and Fig.3C. These are described now.

Fig 4: pEX+NC and pEX CASC2+PTEN shRNA are not significantly different? As they look it, clarifying it with a bar and N.S. might help the reader.
Response: It's significantly different. The figure was corrected now.

8. General remarks
There are a few typos, word repeats and inelegant phrases in the manuscript. A native speaking editor might help by reading over it once or twice.
Response: The English is revised by Nature Research Editing Service (http://bit.ly/NRES-HS).

Reviewer #2:
First of all, language editing is strongly recommended, especially regarding grammar (singular, plural; use of definite and indefinite articles, use of tenses, adverbs and prepositions, and, here and there, wording). As soon as the language in this manuscript has been edited, it should be much easier and more pleasant to read.
Response: The English is revised by Nature Research Editing Service (http://bit.ly/NRES-HS).

Major:
1.Fig. 2 D - F show and compare (pEX+mimics NC), (pEX CASC2+mimics NC), and (pEX CASC2+miR-21 mimics). How would the combined transfection with pEX and just miR-21 mimics (pEX+miR-21 mimics) affect migration and invasion? This is another, what extent miR-21 contributes to cell migration and invasion and (ii) might help the authors to rephrase ll. 25-31 in the abstract :"MiR-21 was a direct target of CACS2. The overexpression of miR-21 significantly abolished the anti-mestastasis effects of CASC2 in PANC-1 cells". Do miR-21 and CASC2 interact directly? In how far does the observation that overexpression of miR-21 abolishes the anti-metastatic effects of CASC2 allow for the conclusion that miR-21 is a direct target of CASC2?
Response: Now, the miR-21 levels in groups including pEX; mimics NC, pEX+mimics NC, pEX+miR-21 mimics, pEX CASC2+mimics NC, pEX CASC2+miR-21 mimics were shown. The migration and invasion in groups including pEX+mimics NC, pEX+miR-21 mimics, pEX CASC2+mimics NC, pEX CASC2+miR-21 mimics were shown. The data showed that MiR-21 mimics significantly promoted migration and invasion, and also significantly reversed the anti-migration and anti-invasion of CASC2 in PANC-1 cells, suggesting overexpression of miR-21 significantly abolished the anti-metastasis of CASC2 in PANC-1 cells.

2.The discussion should be more substantial and elaborate. One third of the discussion (first page of the discussion, l. 40, through second page of the discussion, l. 15, represents just a "renarration" of the results of reference [9] (Yu et al., J Cell Biochem 2017, a DOI number or page numbers are missing). It might help to design a nice figure, a scheme, that shows a chain of events or a regulatory signaling network including the findings of the present and previous studies (also the above-mentioned paper by Yu et al) and to develop the discussion along this scheme. Different colors could be used to differentiate between clear findings and known interactions among RNAs and between RNAs and signaling pathways on the one, and highly probable, yet unknown and therefore hypothetical interactions on the other hand. This could help to structure the discussion and to integrate facts, findings and phenomena observed in the present study, as well as hypothetical ideas being the basis or motivation for future studies.
Response: The discussion is rewritten.

3.Cell migration and invasion/Methods: "After 48 h, the cells on the lower surface were fixED with 4% paraformaldehyde after removal of the cells not migrated or invaded by a cotton swab…., and counted under the microscopE". The authors show and refer to the lower side(s) of the filter(s), but do not include the cells that end up on the bottom of the well after crossing the Matrigel and/or the membrane. As soon as a parameter/condition affects migration and invasion it is potentially able to affect adhesion as well. Consequently, cells that made it through the pore might show differences in adhesiveness depending on the experimental condition/vector/RNA. When less adhesive, they would move across the pore and may then drop to the bottom. When more adhesive they remain "stuck" to the lower side of the filter membrane. Including the cells that dropped to the ground will give the correct number of migrating/invading cells and could even provide information about the grade of adhesiveness at different conditions.
Response: The cells that drop to the bottom can be negligible, so we didn't calculated it.
4.Figs. 1 C,D; 2 E,F; 4 A,B: A scientifically sound micrograph, photograph or image requires a scale bar or a map scale, or at least the magnification needs to be mentioned in the figure legend.
Response: The scale bar is added now.

Minor:
1.There is a confusion/inconsistency with the p-values in the figure legends, the text body, and figures themselves: Figure legend 1 A: "p<0.05. Two asterisks represent p<0.01. In Results, first paragraph, l. 42, it indeed says p<0.01. The end of figure legend 2 reads: "P<0.05; "P<0.01; ""P<0.001." I cannot find a diagram with only one asterisk. Figure legend 3 ends: "P<0.01, but the diagrams are labeled with """ (p<0.001).
Response: "p<0.05 in Figure legend 1A is revised to "P<0.01.
"P<0.05 in Figure 2E and F.
""P<0.01 in Figure 3 is revised to """P<0.001".

2.The numerous typos, errors and syntax errors (far too many to be listed) should be eliminated by language editing.
Response: The English is revised by Nature Research Editing Service (http://bit.ly/NRES-HS).

Reviewer #3:
1, Abstract needs to be rewrite so as to clearly summarize the main stuff of the story.
Response: This is revised.
2, Is it possible that the author could do some animal work to make sure in vivo CASC2 inhibits the growth of pancreatic cancer
Response: This will be studied in the future. The limitation is also described in the main text.
3, Make sure miR-21 is also involved in CASC@-regulated pancreatic tumor growth in vivo if it is not hard for the authors to perform.
Response: The in vivo studies will be studied in the future.

Reviewer #4:
Quality of English language: The manuscript needs to be edited for English language before publication.
Response: English is revised by Nature Research Editing Service (http://bit.ly/NRES-HS).

Additional comments: In the abstract the authors mention that overexpression of CASC2 significantly induced expression of miR-21 but the results suggest that CASC2 downregulates miR-21. So the abstract has to be corrected as it is completely opposite of the results described.
Response: This mistake is corrected now. "CACS2 overexpression inhibited migration and invasion of PAN-1 cells, and significantly inhibited expression of miR-21 and PTEN."

Reviewer #3:
1, Abstract needs to be rewrite so as to clearly summarize the main stuff of the story.
Response: This is revised.
2, Is it possible that the author could do some animal work to make sure in vivo CASC2 inhibits the growth of pancreatic cancer
Response: This will be studied in the future. The limitation is also described in the main text.
3, Make sure miR-21 is also involved in CASC@-regulated pancreatic tumor growth in vivo if it is not hard for the authors to perform.
Response: The in vivo studies will be studied in the future.

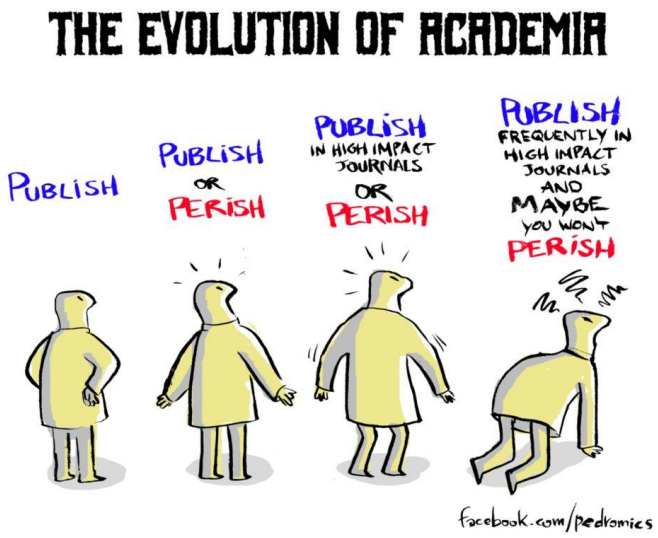
It has become standard to use a mouse model in almost any cancer paper. (personal experience a journal with Impact Factor 2+ asked for mouse data)

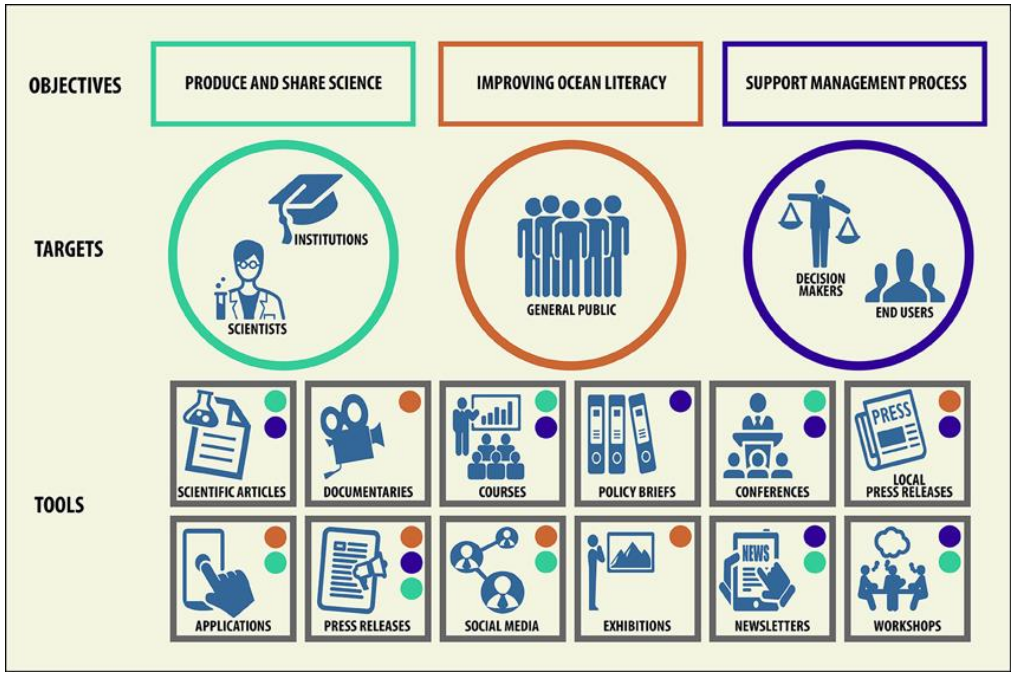
THE '3Rs'

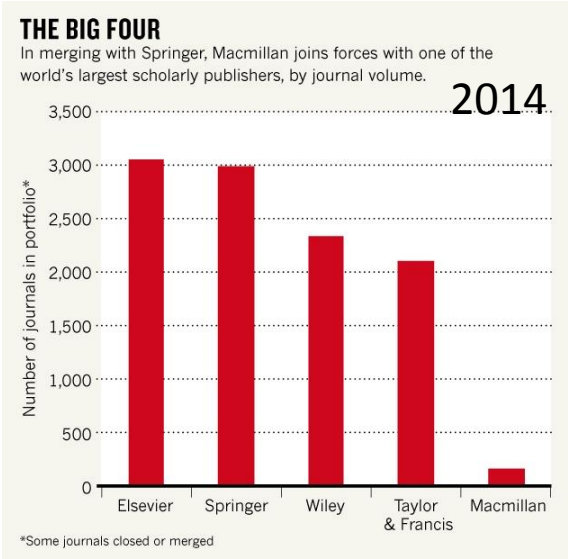
REPLACE the use of animals wherever possible

REDUCE the number of animals needed to a minimum

REFINE tests to cause animals the least possible distress







The 3Rs only work when same rules for everyone

Example provided by Dr Ott for the sake of this discussion

Review Article

Lost in translation: animal models and clinical trials in cancer treatment

Isabella WY Mak[1,2], Nathan Evaniew[1,2], Michelle Ghert[1,2]

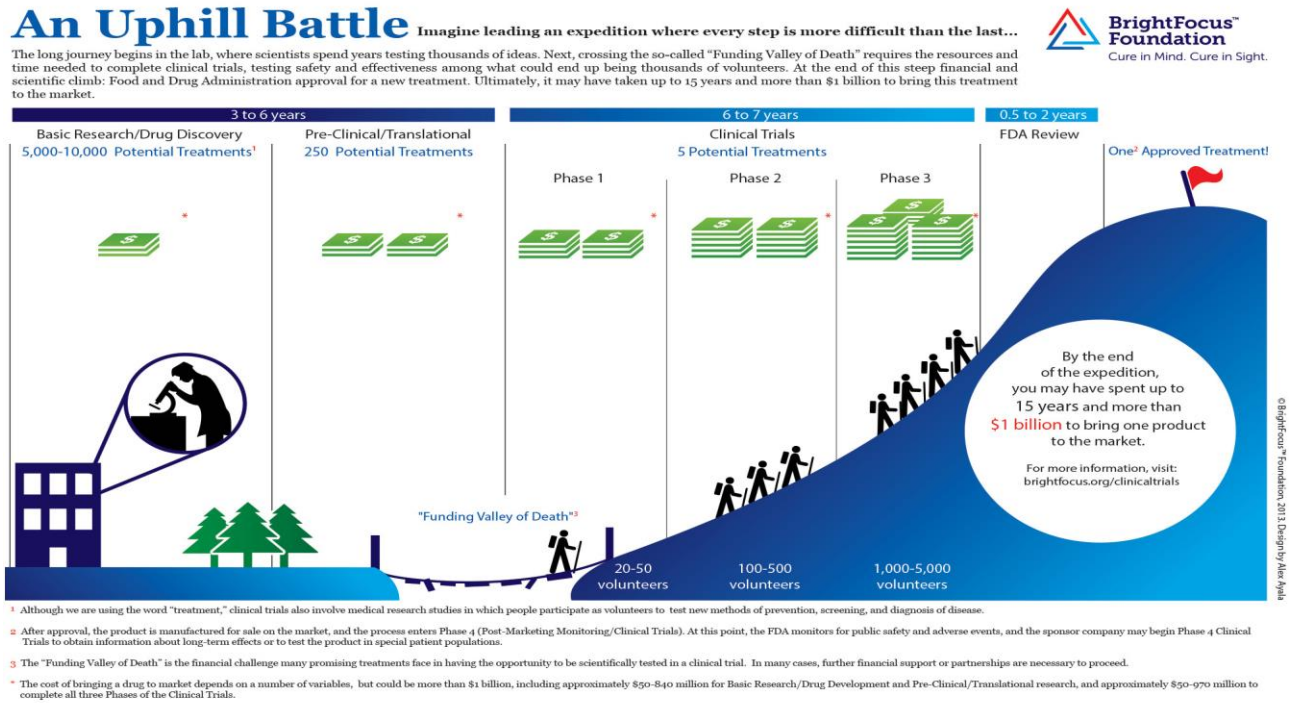
[1]*Department of Surgery, McMaster University, Hamilton, Ontario, Canada;* [2]*Juravinski Cancer Centre, Hamilton Health Sciences, Hamilton, Ontario, Canada*

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.

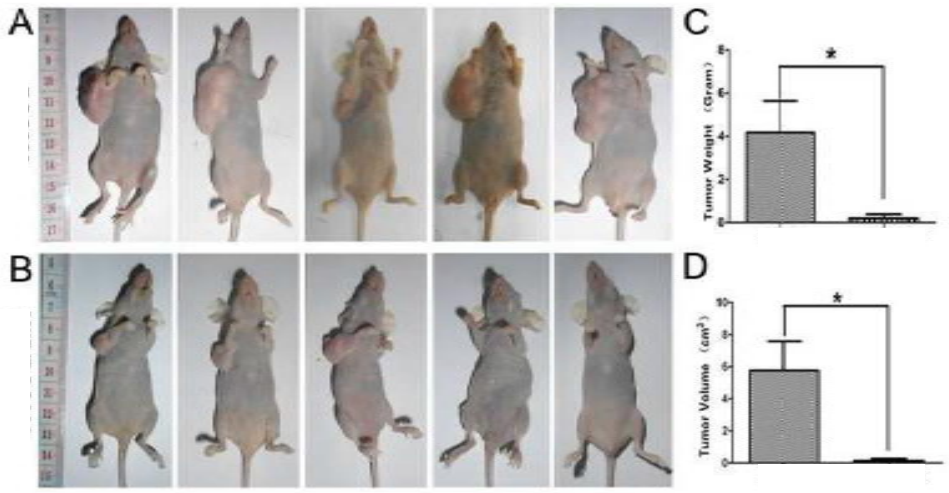
Point 3:

[11] Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. In: Food and Drug Administration: U.S. Department of Health and Human Services; 2004.

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?

What's the point of your model?



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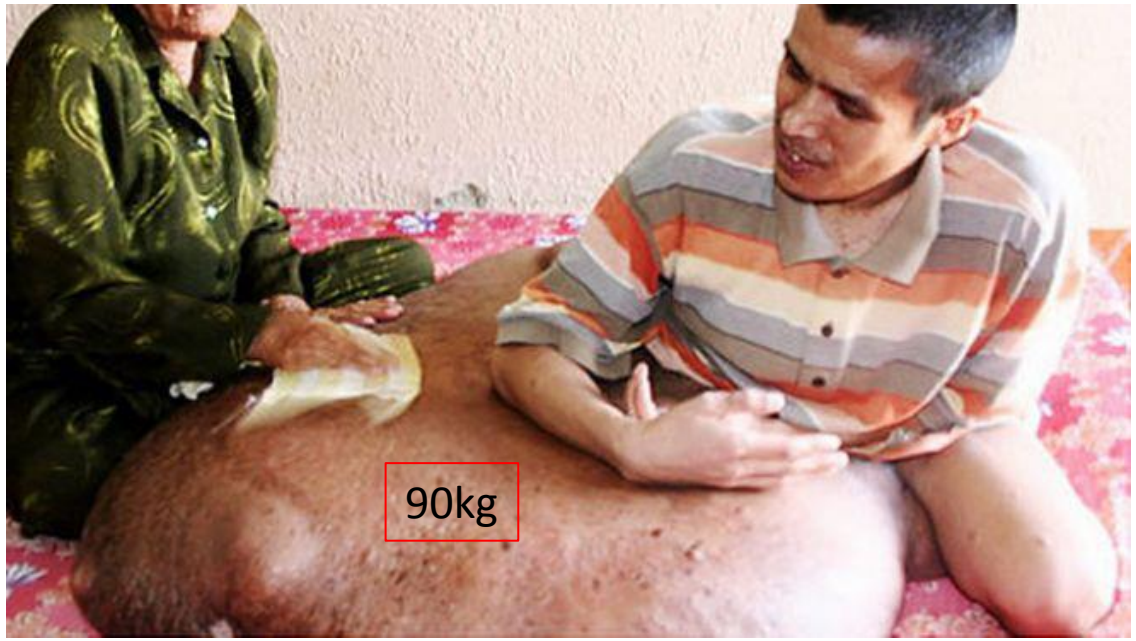


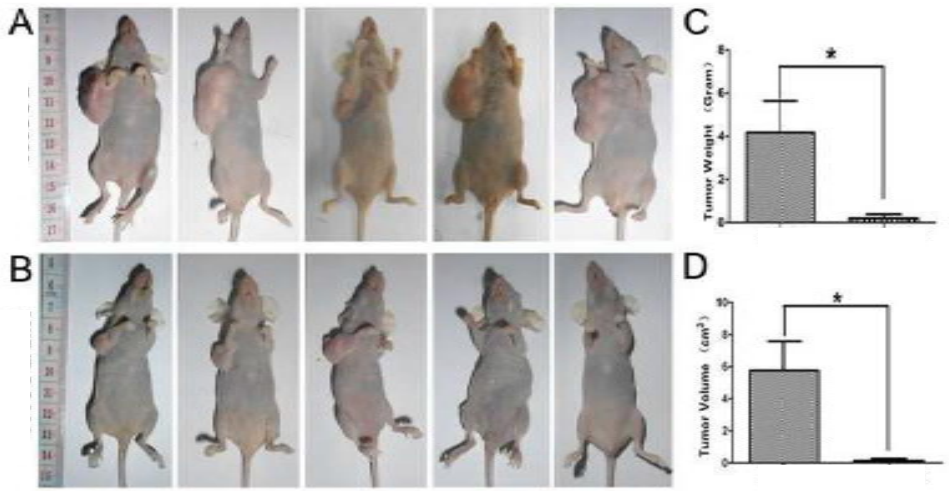
A tumour is considered to be lethal (depending on location and several other factors, such as hormonal secretion) if it reaches a weight of approximately 1 kg, or 10(12) cells, although alternative numbers given suggest a maximum of 10(13) cells.

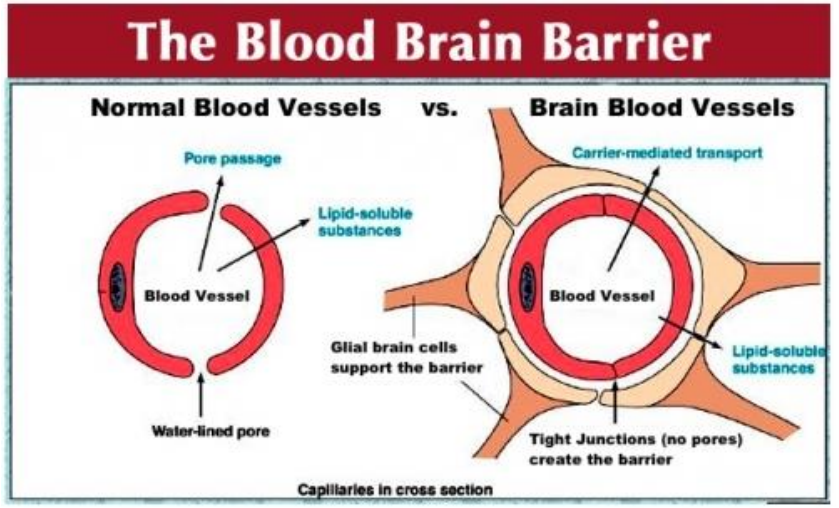
Critical Reviews™ *in Oncogenesis* 21(3-4), 253-267 (2016)

Cell Death Induction in Cancer Therapy—Past, Present, and Future

Lisa Nonnenmacher,[a,*] Sebastian Hasslacher,[a] Julia Zimmermann,[a] Georg Karpel-Massler,[b,c] Katia La Ferla-Brühl,[d] Sara E. Barry,[e] Timo Burster,[c] Markus D. Siegelin,[b] Oliver Brühl,[d] Marc-Eric Halatsch,[c] Klaus-Michael Debatin,[a] & Mike-Andrew Westhoff[a,*]

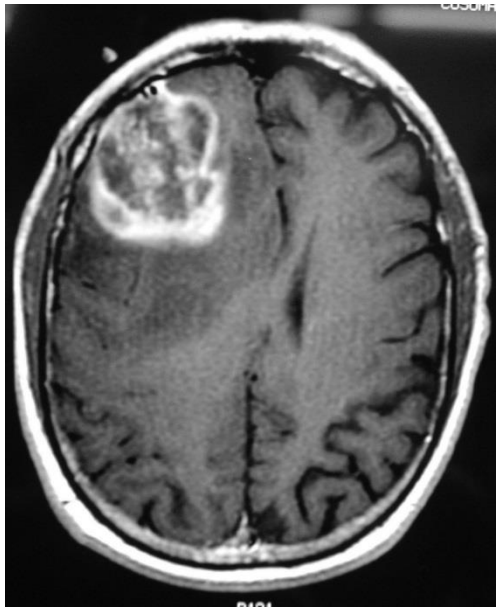
What's the point of your model?





Glioblastoma (GB) overview

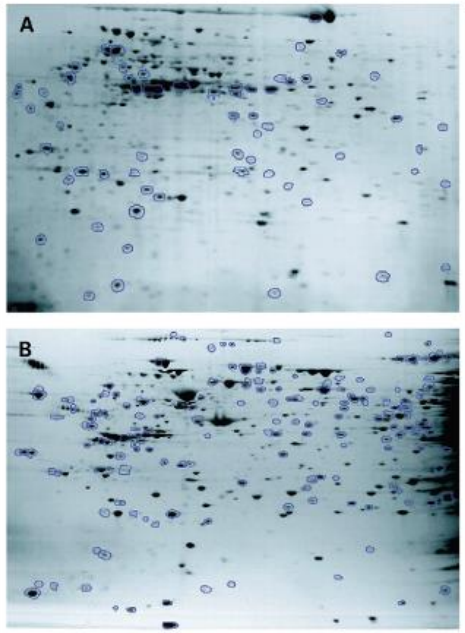
- Most common and aggressive primary brain tumor in adults
- Diffuse and highly invasive growth pattern
- Median patient overall survival: 14.6 months
- Standard therapy consists of
  - Maximal safe surgical resection
  - Radiochemotherapy
  - Chemotherapy (temozolomide)



Clin Cancer Res 2005;11(10) May 15, 2005

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] Weifen Zeng,[1,4,5] Edward H. Oldfield,[1] Alexander O. Vortmeyer,[1] and Robert J. Weil[1,5]

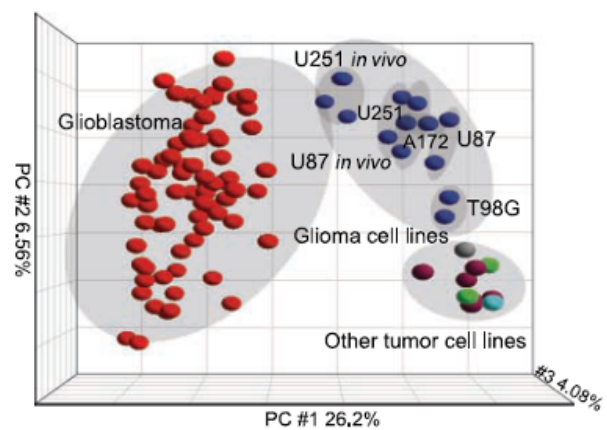


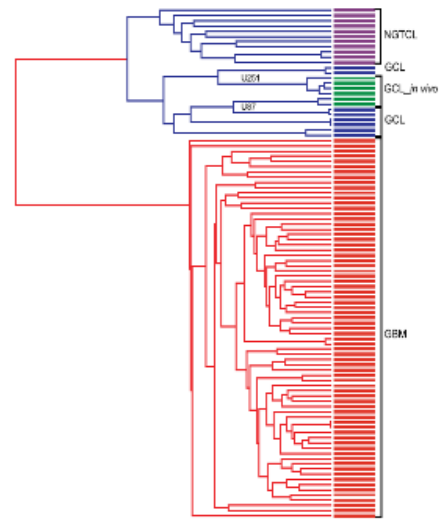
Published OnlineFirst January 9, 2008; DOI:10.1158/1541-7786.MCR-07-0280

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

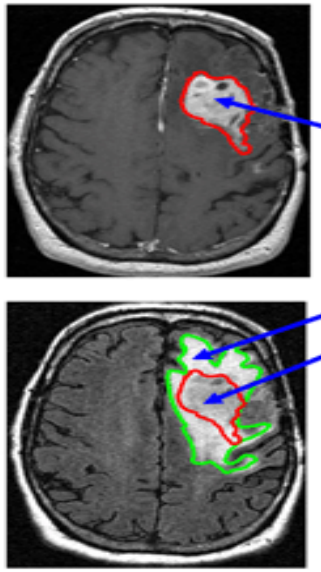
Aiguo Li,[1] Jennifer Walling,[1] Yuri Kotliarov,[1] Angela Center,[1] Mary Ellen Steed,[1] Susie J. Ahn,[1] Mark Rosenblum,[2] Tom Mikkelsen,[2] Jean Claude Zenklusen,[1] and Howard A. Fine[1]

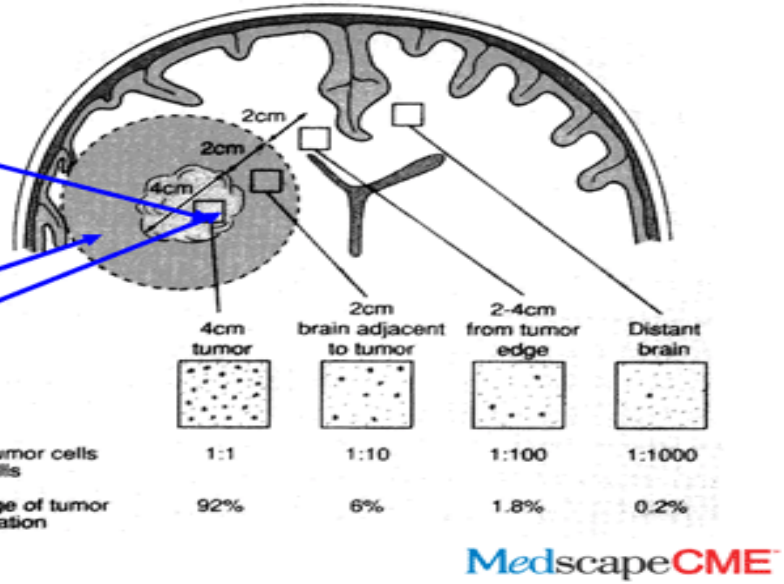
[1]Neuro-Oncology Branch, National Cancer Institute, National Institutes of Neurological Disorder and Stroke, NIH, Bethesda, Maryland and [2]Neurology and Neurosurgery, Hermelin Brain Tumor Center, Henry Ford Hospital, Detroit, Michigan





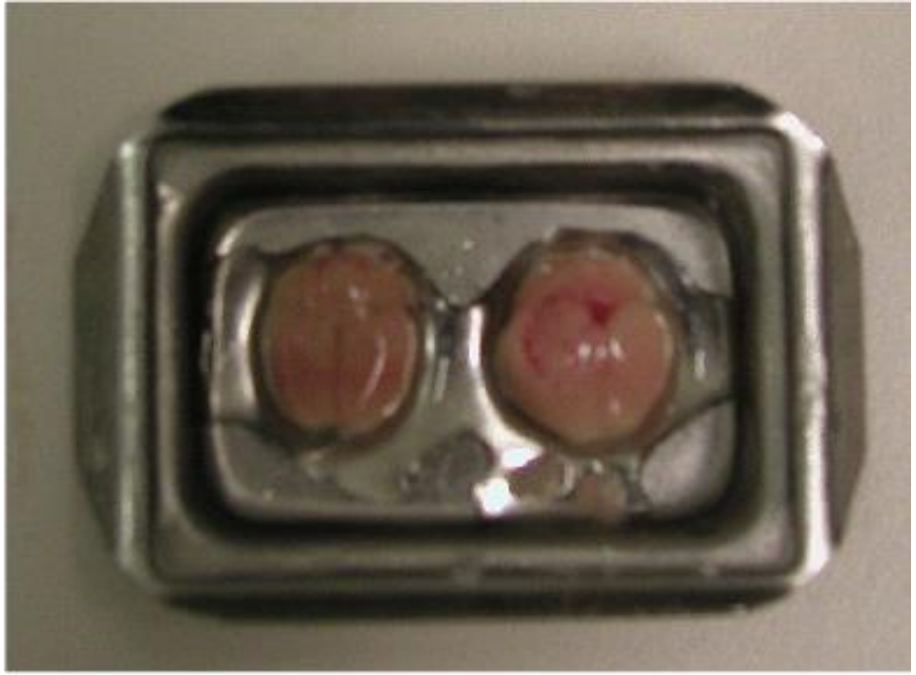
Integrity of BBB: May Vary Within Tumor

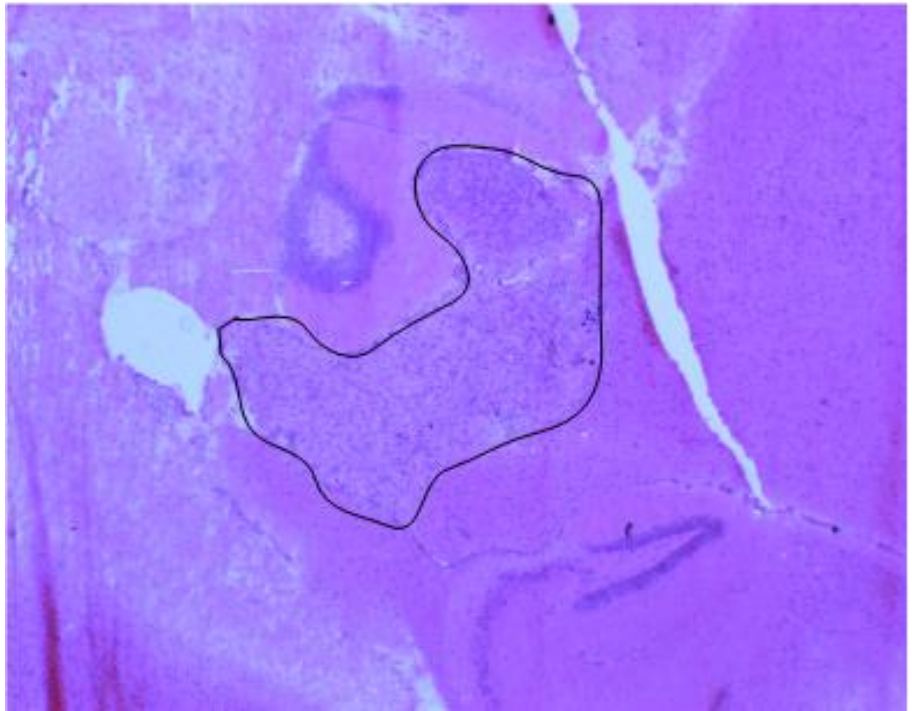


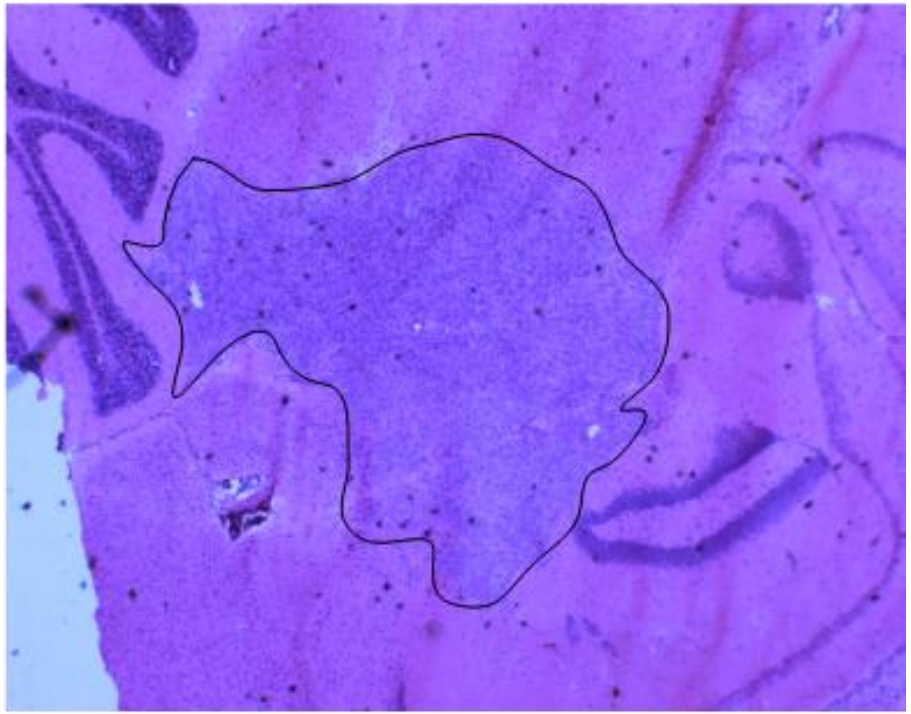


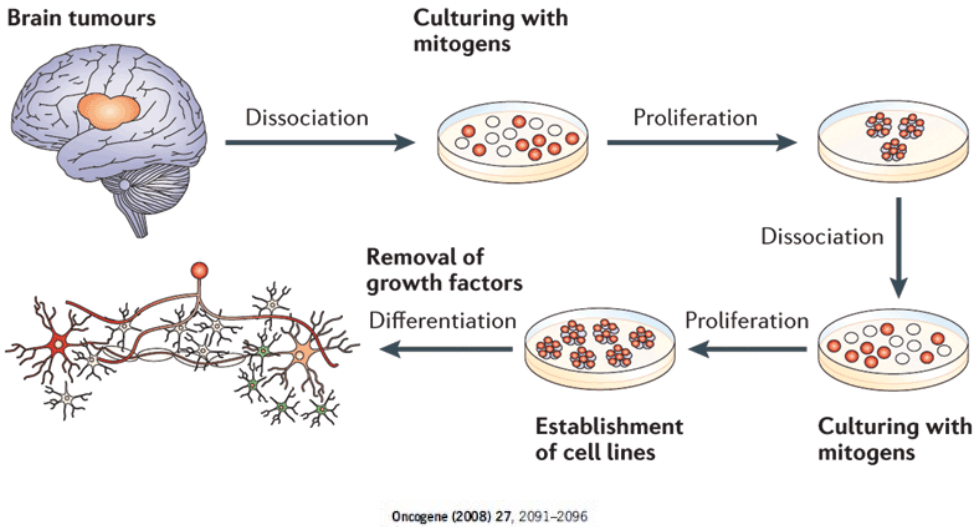
	4cm tumor	2cm brain adjacent to tumor	2-4cm from tumor edge	Distant brain
Ratio of tumor cells to total cells	1:1	1:10	1:100	1:1000
Percentage of tumor cell population	92%	6%	1.8%	0.2%

MedscapeCME





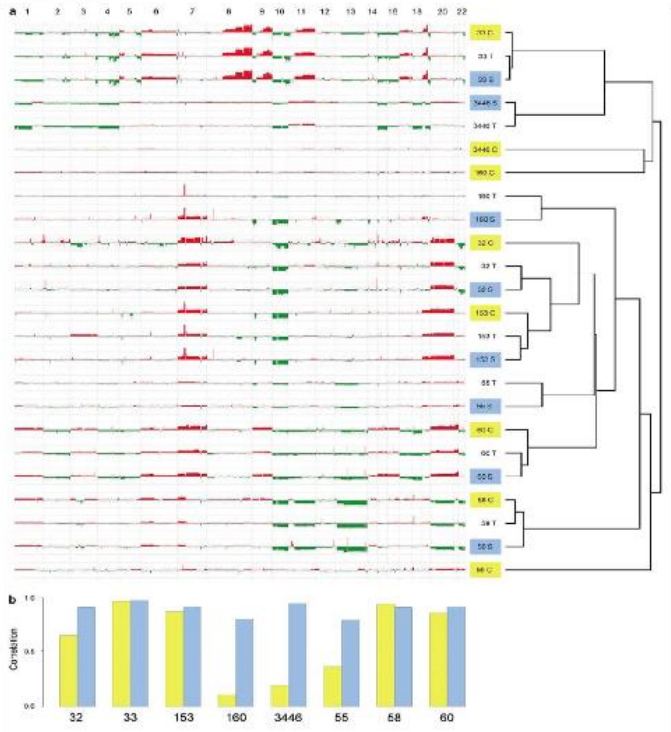


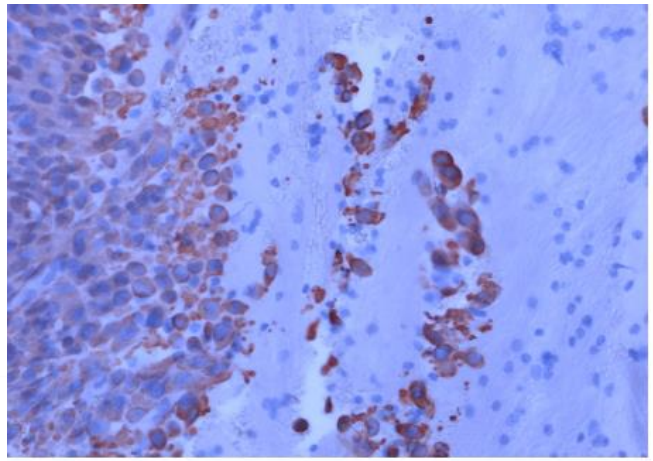


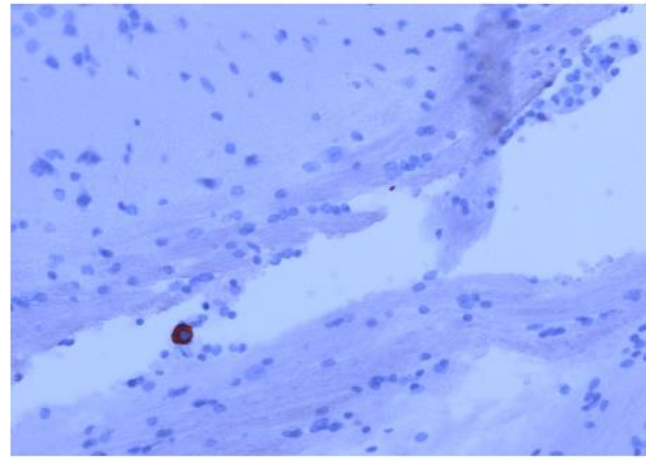
Oncogene (2008) 27, 2091–2096

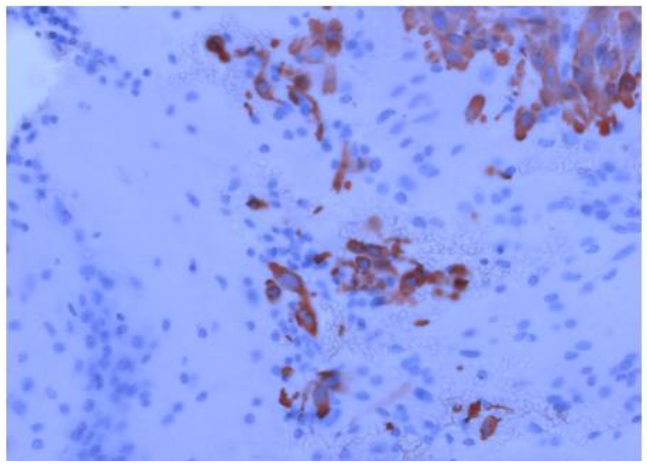
The genomic profile of human malignant glioma is altered early in primary cell culture and preserved in spheroids

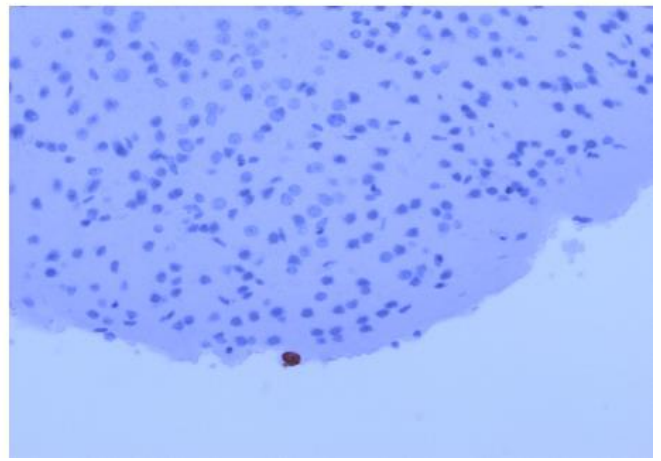
PC De Witt Hamer[1], AAG Van Tilborg[1,2], PP Eijk[3], P Sminia[4], D Troost[2], CJF Van Noorden[5], B Ylstra[3] and S Leenstra[1]











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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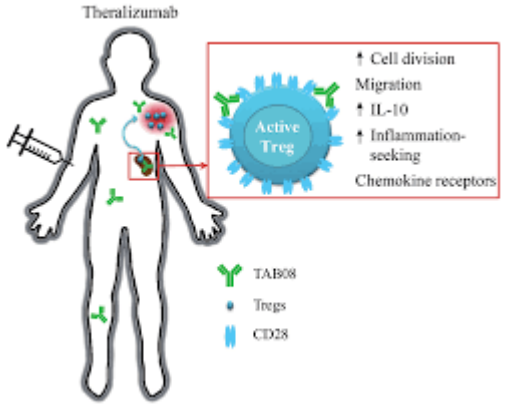
- The targeted drug imatinib (Glivec) can now cure people with chronic myeloid leukaemia. The original studies that identified imatinib's potential were carried out in mice.
- The development of antibody treatments for cancer has also relied on animal research. Antibodies are molecules designed to recognise 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.

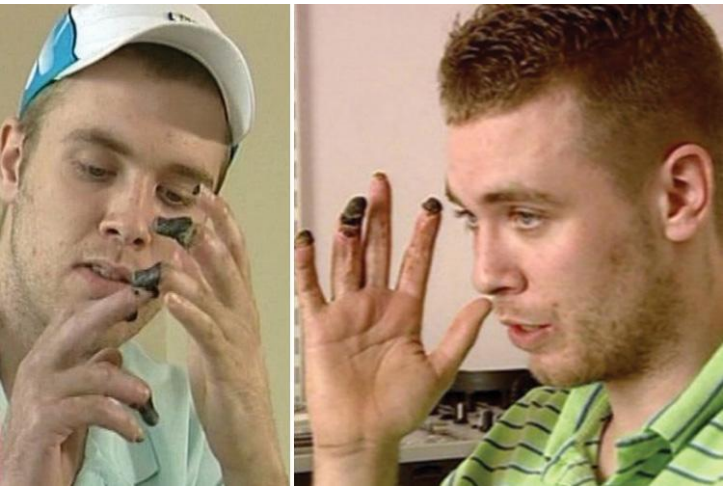
https://scienceblog.cancerresearchuk.org/2011/06/21/animal-research-is-helping-us-beat-cancer/

Theralizumab

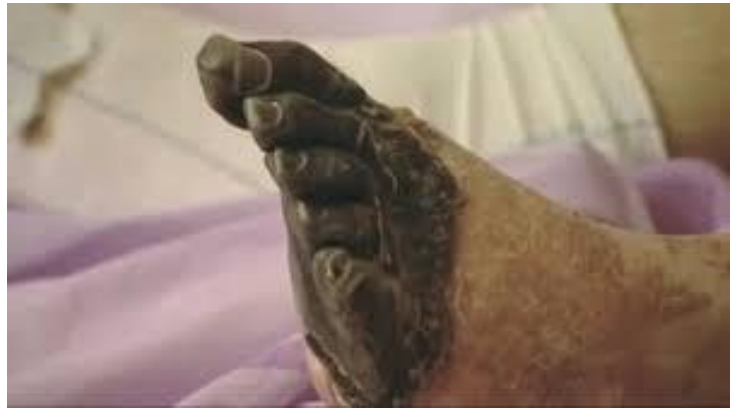
for the treatment of B cell chronic lymphocytic leukemia (B-CLL) and rheumatoid arthritis

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.



Retrieved from "https://en.wikipedia.org/w/index.php?title=Theralizumab&oldid=86006098"3

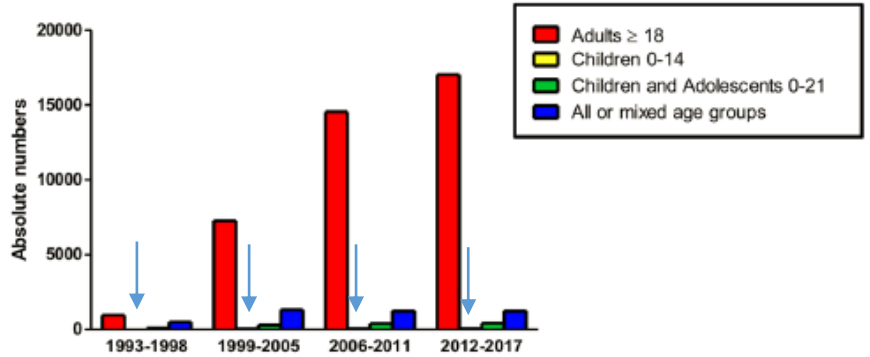
Average # of people joining a clinical trial

1 in 33

adult cancer patients participate in clinical trials.

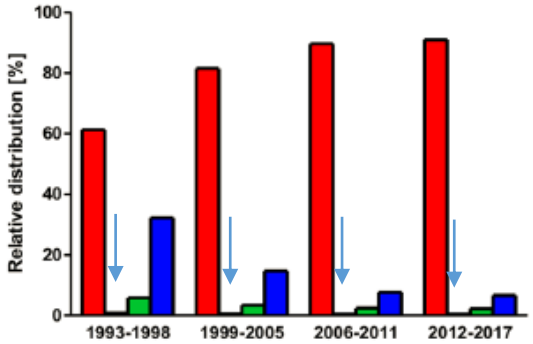
Source from: Institute of Medicine (US) Forum on Drug Discovery, Development, and Translation. Transforming Clinical Research in the United States: Challenges and Opportunities: Workshop Summary. Washington (DC): National Academies Press (US); 2010. 6, Clinical Trials in Cancer. Available from: https://www.ncbi.nlm.nih.gov/books/NBK50895/

A



	Adults ≥ 18	Children 0-14	Children and Adolsecents 0-21	All or mixed age groups
1993-1998	961	11	91	507
1999-2005	7247	42	295	1309
2006-2011	14541	57	392	1232
2012-2017	17018	62	408	1225

B



	Adults ≥ 18	Children 0-14	Children and Adolsecents 0-21	All or mixed age groups
1993-1998	61.21%	0.70%	5.80%	32.29%
1999-2005	81.49%	0.47%	3.32%	14.72%
2006-2011	89.64%	0.35%	2.42%	7.59%
2012-2017	90.94%	0.33%	2.18%	6.55%

Fig. 3 Changes over time of clinical trials grouped according to age of subjects. .Interventional cancer-related clinical studies registered at ClinicalTrials.gov were categorised into four distinct groups, indicating whether the subjects were children, children and adolescents, adults or mixed (each study was allocated 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)

OPINION

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**

Aberration	Diagnosis frequency (%) ($n = 240$)[22]	Diagnosis frequency (%) ($n = 230$)[24]	Relapse frequency (%)[a] ($n = 59$)[27]
ALK mutation	9.2	14.3	24.7
PTPN11 mutation	2.9	1.3	0
ATRX mutation	2.5	1.8	11.1
ATRX deletion	7.1	4.0	5.6
MYCN mutation	1.7	0.9	3.7
MYCN amplification	32.0	25.7	18.5
NRAS mutation	0.8	2.6	7.4
NF1 mutation or truncation	0	2.2	5.6
KRAS mutation	0	1.7	1.9
FGFR1 mutation	0	1.7	9.3
TP53 mutation	0.8	3.5	7.4

[a]Includes variants of unknown significance.

10.3 per 1M children
Neuroblastoma a copy number disease
Relapse a mutational disease, more drugable targets

VOLUME 26 · NUMBER 2 · JANUARY 10 2008

JOURNAL OF CLINICAL ONCOLOGY

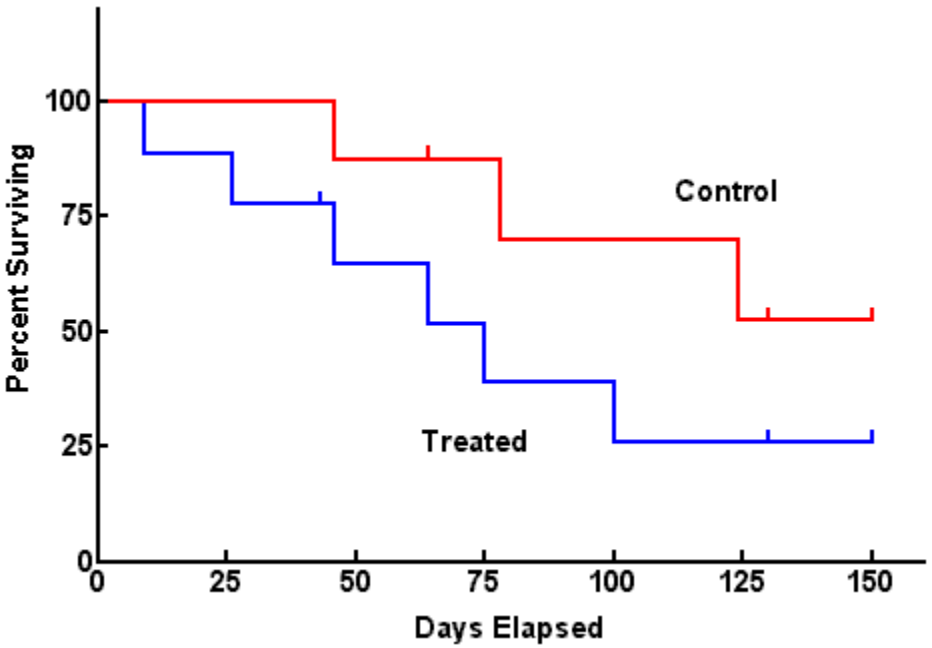
ORIGINAL REPORT

Shortening the Timeline of Pediatric Phase I Trials: The Rolling Six Design

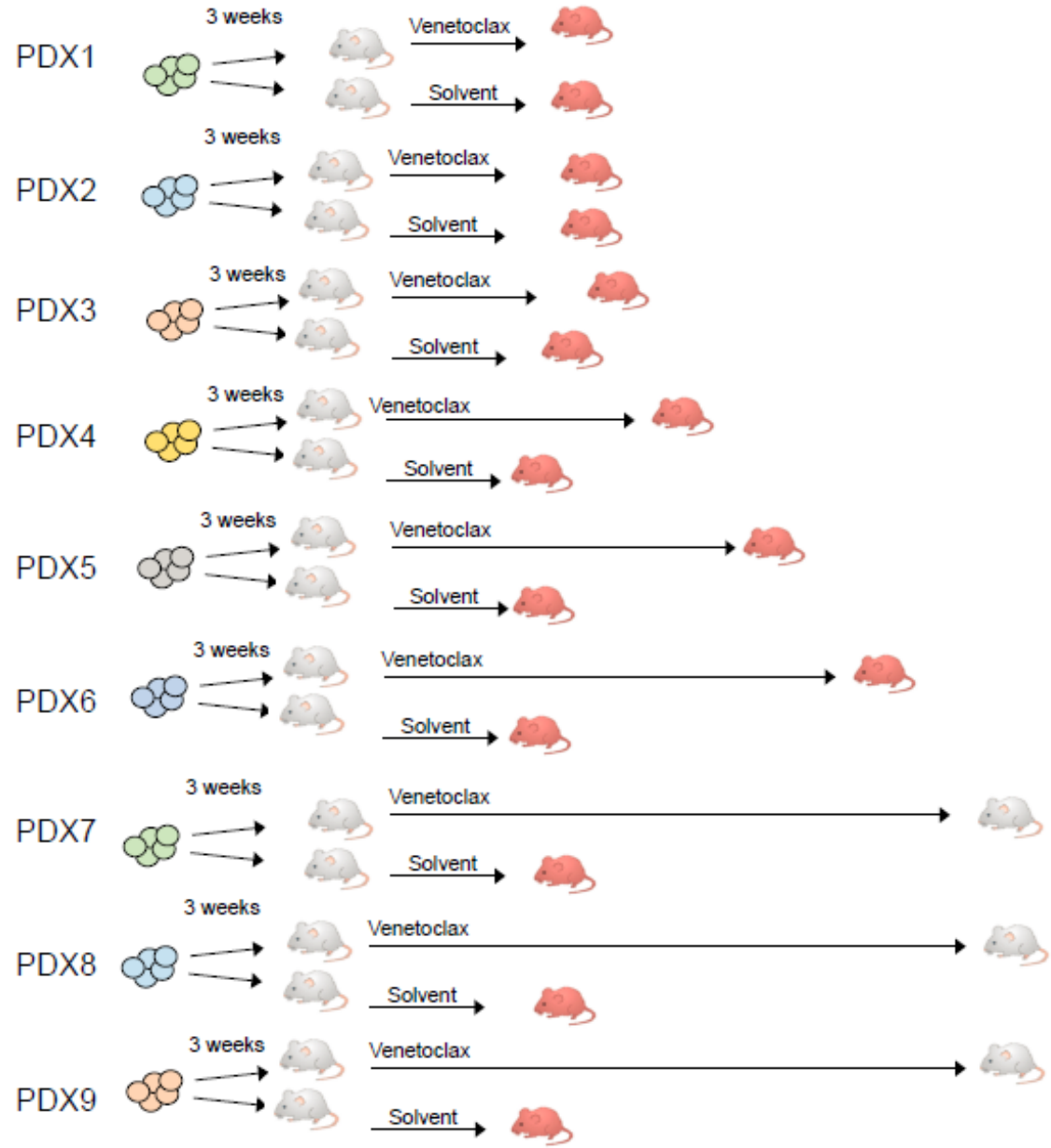
Jeffrey M. Skolnik, Jeffrey S. Barrett, Bhuvana Jayaraman, Dimple Patel, and Peter C. Adamson

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.


Percent Surviving
100
75
50
25
0
0
25
50
75
100
125
150
Days Elapsed
Control
Treated

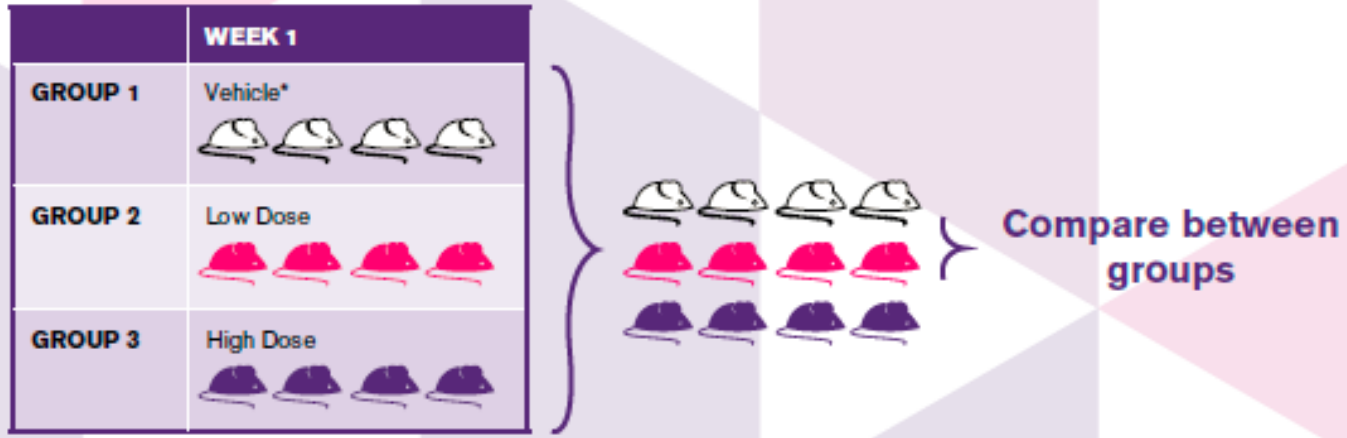
versus


PDX1
3 weeks
Venetoclax
Solvent
PDX2
3 weeks
Venetoclax
Solvent
PDX3
3 weeks
Venetoclax
Solvent
PDX4
3 weeks
Venetoclax
Solvent
PDX5
3 weeks
Venetoclax
Solvent
PDX6
3 weeks
Venetoclax
Solvent
PDX7
3 weeks
Venetoclax
Solvent
PDX8
3 weeks
Venetoclax
Solvent
PDX9
3 weeks
Venetoclax
Solvent

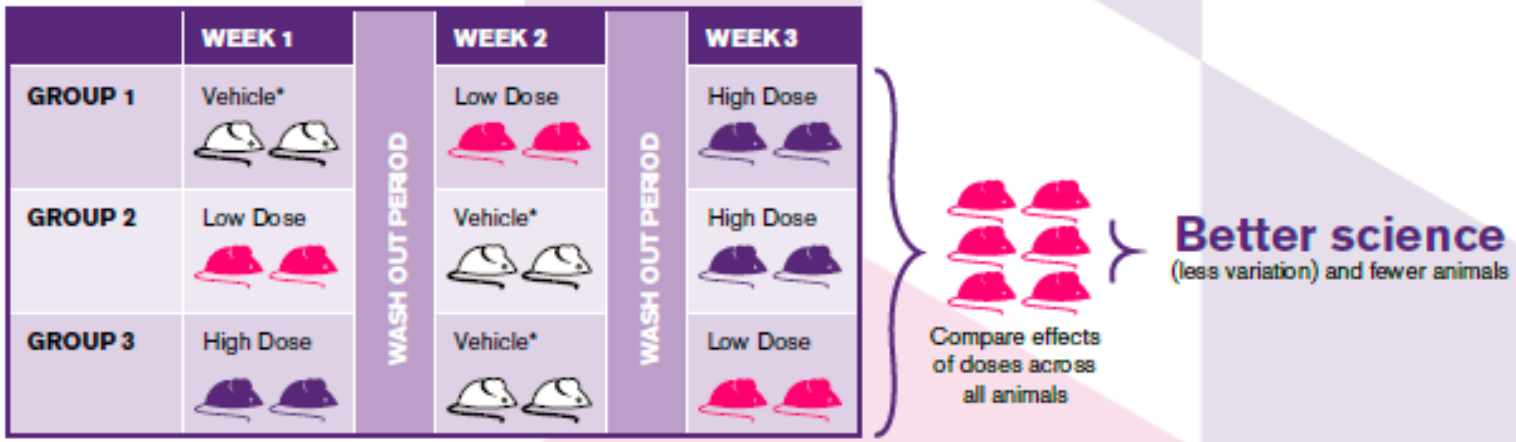
Pharmacokinetic studies redesigned

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



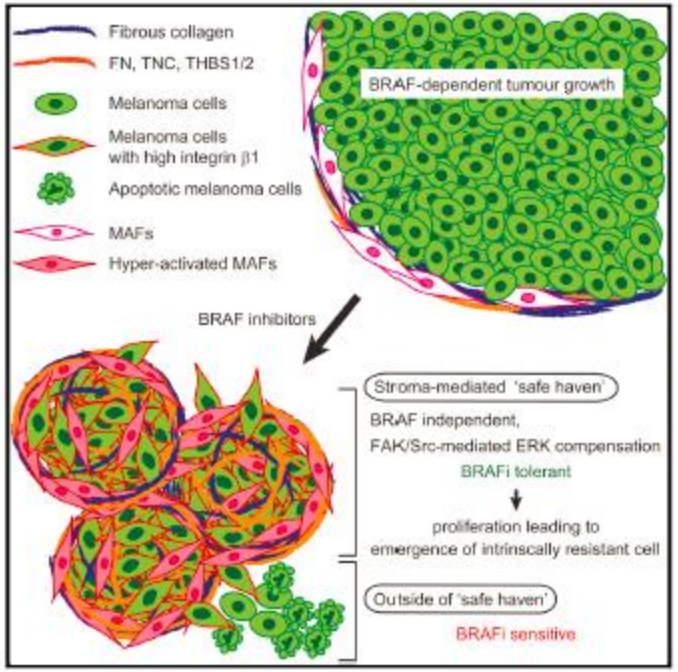
*Vehicle is the solution in which the drug is dissolved. It may be water or another solvent which has minimal effects on the animal. One group is given the vehicle alone, without drug, as a control group.

Association of the British Pharmaceutical Industry

Cancer Cell

Intravital Imaging Reveals How BRAF Inhibition Generates Drug-Tolerant Microenvironments with High Integrin β1/FAK Signaling

Graphical Abstract



Authors

Eishu Hirata, Maria Romina Girotti, ..., Richard Marais, Erik Sahai

Correspondence

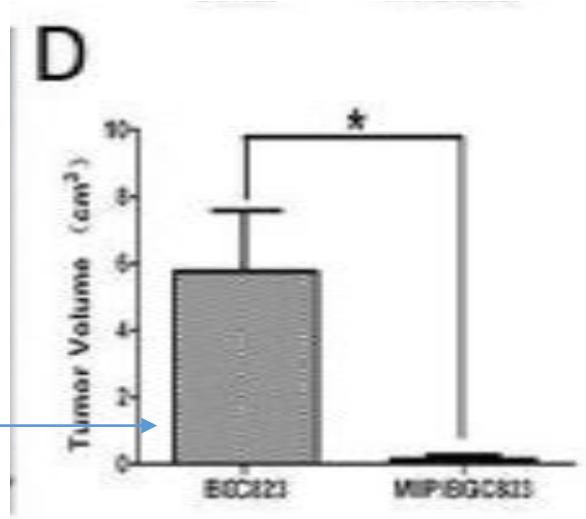
erik.sahai@crick.ac.uk

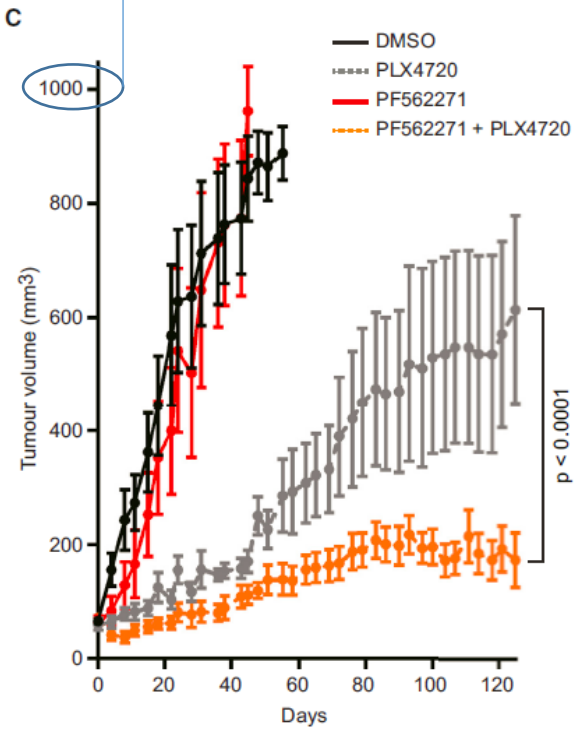
In Brief

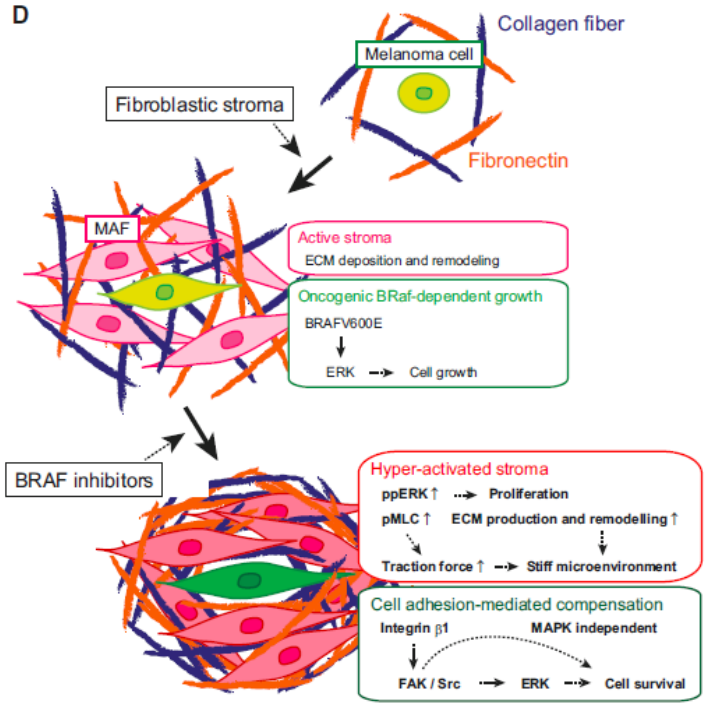
Hirata et al. show that the BRAF inhibitor PLX4720 promotes melanoma-associated fibroblasts in *BRAF*-mutant melanomas to produce and remodel matrix, leading to integrin β1-FAK-Src signaling and reactivation of ERK and MAPK in melanoma cells. Co-inhibition of BRAF and FAK blocks ERK reactivation.

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





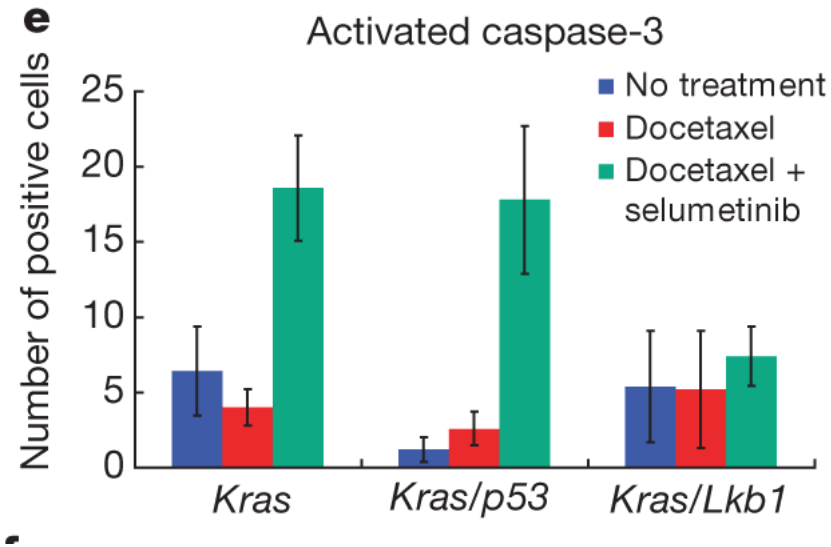


Nature. ; 483(7391): 613–617. doi:10.1038/nature10937.

A murine lung cancer co–clinical trial identifies genetic modifiers of therapeutic response

Zhao Chen[1,2,3], **Katherine Cheng**[2,3], **Zandra Walton**[2,3], **Yuchuan Wang**[4,5,6], **Hiromichi Ebi**[1,7], **Takeshi Shimamura**[8], **Yan Liu**[1,2,3], **Tanya Tupper**[4], **Jing Ouyang**[2], **Jie Li**[9], **Peng Gao**[2,3], **Michele S. Woo**[2], **Chunxiao Xu**[1,2,3], **Masahiko Yanagita**[2], **Abigail Altabef**[2], **Shumei Wang**[10], **Charles Lee**[10], **Yuji Nakada**[11], **Christopher G. Peña**[11], **Yanping Sun**[4,5], **Yoko Franchetti**[12], **Catherine Yao**[2], **Amy Saur**[4], **Michael D. Cameron**[13], **Mizuki Nishino**[5,6], **D. Neil Hayes**[14], **Matthew D. Wilkerson**[14], **Patrick J. Roberts**[14], **Carrie B. Lee**[14], **Nabeel Bardeesy**[7], **Mohit Butaney**[2], **Lucian R. Chirieac**[10], **Daniel B. Costa**[15], **David Jackman**[2], **Norman E. Sharpless**[14], **Diego H. Castrillon**[11], **George D. Demetri**[3], **Pasi A. Jänne**[1,2,16], **Pier Paolo Pandolfi**[17], **Lewis C. Cantley**[18,19], **Andrew L. Kung**[4,20], **Jeffrey A. Engelman**[1,7], and **Kwok-Kin Wong**[1,2,3,16]

e

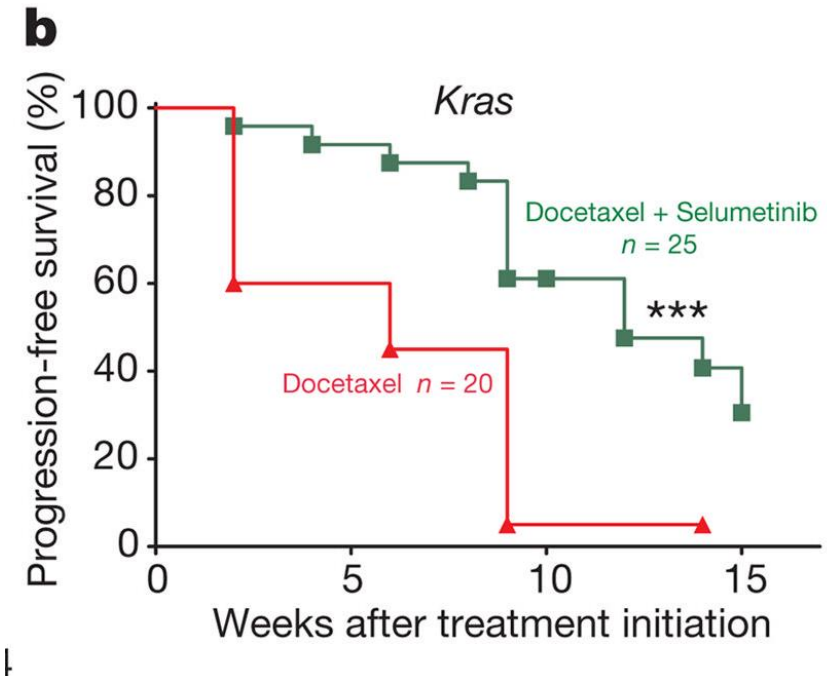


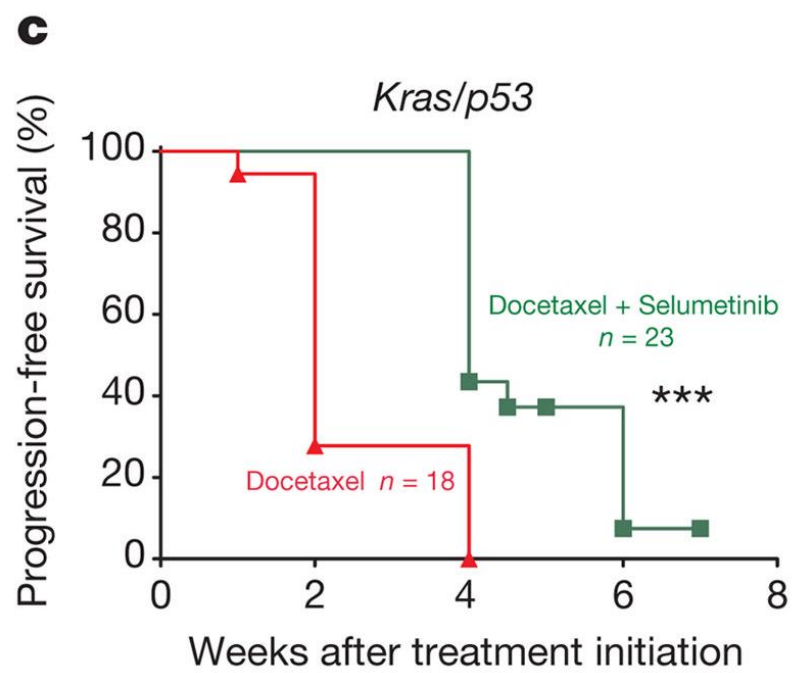
c

Treatment	Genotype	Partial response % (>30% regression)	Stable disease %	Disease progression % (>30%)
Docetaxel	*Kras*	30	55	15
Selumetinib + docetaxel	*Kras*	92	4	4
Docetaxel	*Kras/p53*	5	28	67
Selumetinib + docetaxel	*Kras/p53*	61	39	0
Docetaxel	*Kras/Lkb1*	0	78	22
Selumetinib + docetaxel	*Kras/Lkb1*	33	50	17

d

Mutation status	Number of patients	Average pERK score
WT	33	0.74
LKB1	1	1
p53	15	0.4
LKB1/p53	1	0
KRAS	2	0.75
KRAS/LKB1	2	0.5
KRAS/p53	3	2.2


b
Kras
Progression-free survival (%)
100
80
60
40
20
0
0
5
10
15
Weeks after treatment initiation
Docetaxel + Selumetinib
n = 25
***
Docetaxel n = 20


c
Kras/p53
Progression-free survival (%)
100
80
60
40
20
0
0
2
4
6
8
Weeks after treatment initiation
Docetaxel + Selumetinib
n = 23
***
Docetaxel n = 18

LMO1 Synergizes with MYCN to Promote Neuroblastoma Initiation and Metastasis

Shizhen Zhu,[1,12,14,*] Xiaoling Zhang,[1,12] Nina Weichert-Leahey,[2,13] Zhiwei Dong,[1,13] Cheng Zhang,[3] Gonzalo Lopez,[4] Ting Tao,[2] Shuning He,[2] Andrew C. Wood,[5] Derek Oldridge,[4] Choong Yong Ung,[3] Janine H. van Ree,[1] Amish Khan,[1] Brittany M. Salazar,[1] Edroaldo Lummertz da Rocha,[3] Mark W. Zimmerman,[2] Feng Guo,[2] Hong Cao,[1] Xiaonan Hou,[6] S. John Weroha,[6] Antonio R. Perez-Atayde,[7] Donna S. Neuberg,[8] Alexander Meves,[9] Mark A. McNiven,[1] Jan M. van Deursen,[1] Hu Li,[3] John M. Maris,[4,10,11] and A. Thomas Look[2,*]

[1]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Mayo Clinic Cancer Center, Rochester, MN 55902, USA

[2]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA

[3]Department of Molecular Pharmacology & Experimental Therapeutics, Center for Individualized Medicine, Mayo Clinic College of Medicine, Rochester, MN 55902, USA

[4]Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

[5]Department of Molecular Medicine, University of Auckland, Auckland, New Zealand

[6]Departments of Oncology, Radiation Oncology, and Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN 55902, USA

[7]Department of Pathology, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA

[8]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[9]Department of Dermatology, Mayo Clinic, Rochester, MN 55902, USA

[10]Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

[11]Abramson Family Cancer Research Institute, Philadelphia, PA 19104, USA

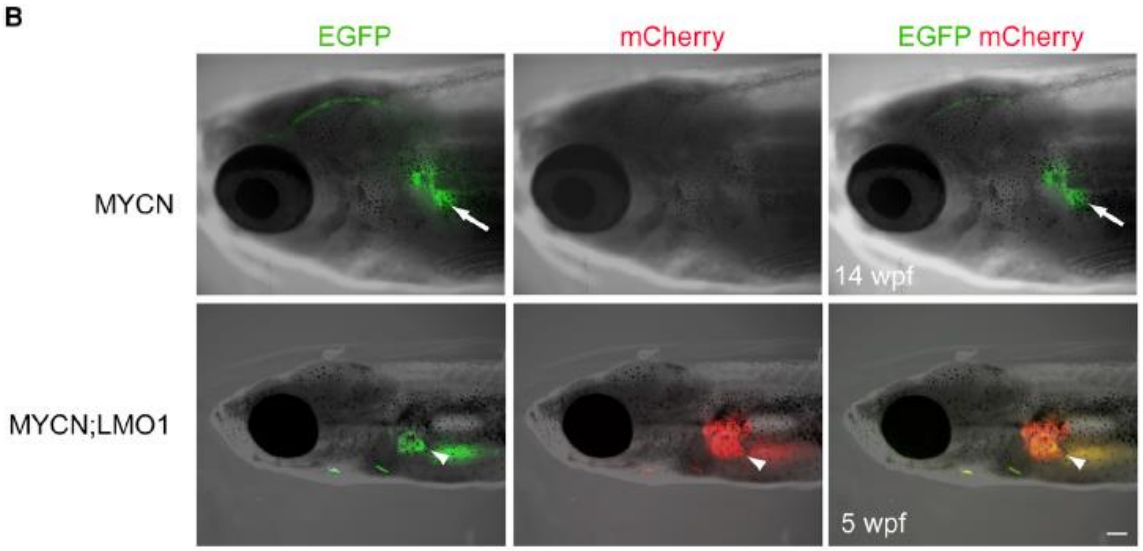
[12]These authors contributed equally

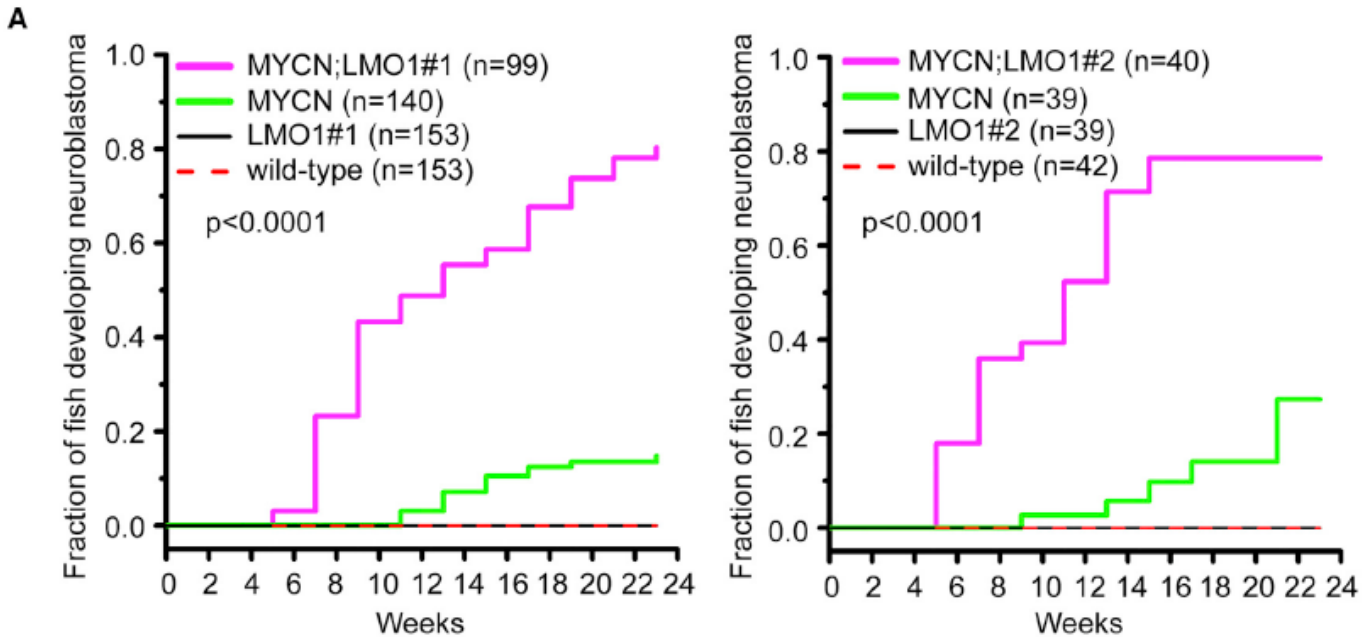
[13]These authors contributed equally

[14]Lead Contact

*Correspondence: zhu.shizhen@mayo.edu (S.Z.), thomas_look@dfci.harvard.edu (A.T.L.)

http://dx.doi.org/10.1016/j.ccell.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

Gonzalez C. Drosophila melanogaster: a model and a tool to investigate malignancy and identify new therapeutics. *Nature Reviews Cancer* 13:172.

nature COMMUNICATIONS

Impact Factor 12.353 (2017)

ARTICLE

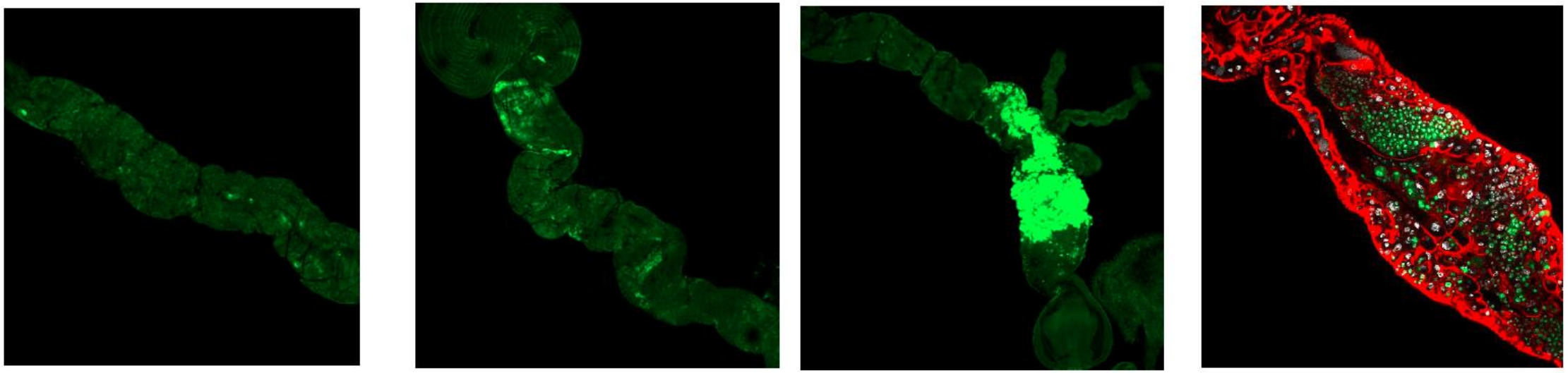
DOI: 10.1038/s41467-018-05737-w OPEN

Social environment mediates cancer progression in *Drosophila*

Erika H. Dawson[1,2], Tiphaine P. M. Bailly[1], Julie Dos Santos[1], Céline Moreno[1], Maëlle Devilliers[3], Brigitte Maroni[3], Cédric Sueur[4,5], Andreu Casali[6], Beata Ujvari[7], Frederic Thomas[8], Jacques Montagne[3] & Frederic Mery[1]

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



(A) 7 days post induction

(B) 14 days post induction

(C) 21 days post induction

(D) 24 days post induction

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

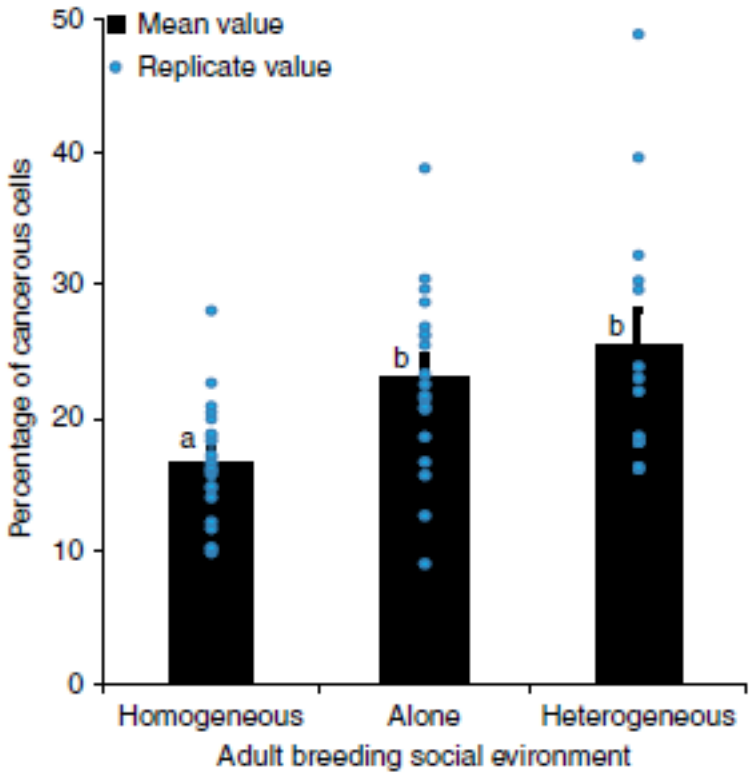


Fig. 1 Gut tumor progression as a function of social environment. FACS analysis of GFP-positive cells in guts dissected from 21 days post-HS cancerous females as a function of social environment. Blue dots indicate mean value for each replicate. Error bars: standard error of the mean. $N =$ 15 measures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

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: $p<0.05$?

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.