HUNTSMAN CANCER INSTITUTE
UNIVERSITY OF UTAH

Joshua D. Schiffman, MD
Huntsman Cancer Institute
University of Utah
2000 Circle of Hope
Salt Lake City, Utah 84112
Phone: (801) 587-4745
Fax: (801) 585-6410

June 15, 2016

Howard Bauchner, MD
Editor in Chief, *JAMA*
Re: PETA's request for retraction

Dear Dr. Bauchner and JAMA editorial staff:

Thank you for sharing the recent letter from PETA (People for the Ethical Treatment of Animals) Foundation and their concern about the scientific validity of our publication on the potential molecular mechanisms for cancer resistance in elephants and the comparative cellular response to DNA damage in humans. Like JAMA, scientific integrity remains of the utmost priority to our research group and we appreciate the opportunity to respond. We will address each point raised by PETA and explain the scientific accuracy of our findings.

PETA has expressed concerns that our calculations for the incidence of cancer in elephants are unreliable and therefore contribute to misleading conclusions. We strongly disagree with this statement for a number of reasons. PETA's concerns are based on the fact that part of our study included a calculation of cancer incidence in captive elephants and this may not reflect the rate and causes of death in wild elephants. Although we appreciate Dr. Rally's concern on behalf of PETA, these issues were carefully addressed 1) in the text of the article, 2) the online supplemental section, and 3) a published response to a letter-to-the-editor. As you know very well, the JAMA peer-review and editorial process is extremely rigorous and prior to this manuscript's acceptance we submitted no less than three revisions with over 130 individual responses to reviewer questions to insure the accuracy, validity, and implications of our scientific findings. The issue of captivity was brought up by the reviewers and, in fact, the discussion section was revised several times to explicitly address both the reliability and limitations of using captive elephant data to support the known observation of limited cancer in elephants. With guidance from JAMA, we were very cautious in the interpretation of the data. Therefore, PETA should be reassured that we have appropriately and reasonably addressed the reliability of using captive animal data for the JAMA readers.

In the published article's Discussion Section, we write: **"The cross-species mortality rates in this study included estimates based on small numbers of captive animals with wide confidence intervals. More data need to be collected to confidently demonstrate the absence of correlation of mass and life span with cancer mortality. Environmental factors also play a role in cancer development, and it is unclear how captivity influences cancer rates through diet, stress, physical activity, and reproduction. The expected life span of captive African and Asian elephants is decreased,[40] and this analysis may not have fully captured the elderly elephant population most expected to develop cancer. Adding to the complexity, humans are treated with modern medicine and may have an artificially extended life span, which, along with carcinogenic exposures like smoking, increases the lifetime risk of cancer death."**

In the Supplement, we note the specific steps taken to analyze the data set from captive elephants, including the age-adjusted lifetime risk: **"Causes of death were divided into seven categories: cancer, euthanized because of cancer, non-cancer disease, euthanized for a reason other than cancer, unspecified disease, euthanized for an unspecified reason, and exogenous cause of mortality. Exogenous causes of mortality include accidents (e.g. falling in the enclosure) and animal fights that cause fatal injury. Inferred cancer rates were calculated by assuming the same percentage of deaths with an unknown cause would be due to cancer as deaths with known causes. For example, if cancer makes up *x* percent of deaths with a known cause, then it was assumed that cancer was also responsible for *x* percent of the deaths with an unspecified cause (i.e. "disease unspecified" and "euthanized unspecified").**

Specifically, the fraction of cancers reported in deaths with a specified disease was f_{dk} and the fraction of elephant euthanizations attributed to cancer is f_{ek}, where the subscript *k* represented 'known' and the *d* and *e* represented 'disease' and 'euthanized' respectively. The number of deaths from unspecified diseases that can be inferred to be cancer was equal to $f_{dk} \times N_{du}$, where N_{du} was the number of deaths caused by an unspecified disease. Similarly the number of unspecified euthanizations that were estimated due to cancer was equal to $f_{ek} \times N_{eu}$, where N_{eu} was the number of euthanizations with no specified reason. The ceiling integer for each of these values was taken as a conservative measure of the cancer incidence. The inferred cancer rate was equal to $\frac{(f_{dk} \times N_{du}) + (f_{ek} \times N_{eu}) + C_{dk} + C_{ek}}{N}$, where C_{dk} and C_{ek} were the number of cancer cases in the known disease population and the known euthanized population respectively and *N* was the total number of elephant deaths. 95% confidence intervals were calculated with the standard error (observed cancer % ± 1.96 x SE, were SE is the standard error). One pseudo-count was added to both the tumor positive and tumor negative observations for each species and the standard error was calculated as $SE = \sqrt{p(1-p)/n}$ where n is the number of necropsies (including the 2 pseudo-counts) and p is the proportion of necropsies with a tumor (including pseudo-counts).

In this study, a simplified estimation of cancer mortality rates was used due to the lack of detailed information in elephant populations. If the standard age population structure of elephants was better understood and one could obtain well documented cancer mortality rates per year (requiring a much larger sample size), then the estimates would be more comparable to those calculated for humans by GLOBOCAN and the American Cancer Society (ACS). To make as realistic as possible estimates given the available data for elephants, the inferred mortality rates were calculated to account for the unknown causes of death as described above. Additionally, the age-adjusted lifetime risk was calculated by weighting each age-specific cancer death rate by the proportion of the population in the given age group and summing these for the cumulative risk. This is based on the methods used for human data[4,5]; however, there was not a standard population structure available to use for elephants so the distribution of ages found in this population of 644 deaths was applied. The age-adjusted lifetime risk was not statistically different from the crude calculation of lifetime risk of cancer mortality in elephants (4.81% vs 4.82%). As more data becomes available, these cancer mortality rates can be refined to enable a more direct comparison with human cancer mortality rates."

In our letter-to-the-editor reply, we also address the issue of interpreting captive animal data: **"One of the limiting factors in comparative oncology is the lack of good data on cancer in both wild and captive animals. As *Pessier et al.* describe, the lay database of elephant deaths that we used may contain biases and we welcome the extensive expertise and careful data curation by the San Diego Zoo for elephants and other animals. *Pessier et al.* report that 2 of their 12 (16.67%) San Diego Zoo elephants died of cancer (95% Confidence Interval [CI]: 0%–37.75%) consistent with our estimate of 4.8% elephant cancer mortality.[1] *Pessier et al.* highlight the large number of benign uterine leiomyomas and malignant uterine tumors in their elephants; this high prevalence of uterine tumors has been correlated with nulliparous status in captive elephants,[2,3] rhinoceroses,[3] and even ovariectomized guinea pigs.[4] Disrupted life history strategies in humans also have been associated with increased reproductive cancer risk (e.g., reduced parity and limited breast feeding with estrogen-positive breast cancer[5] and nulliparity, regardless of fertility, with endometrial cancer[6]). Genomic analysis for *TP53* mutations or deletions in the San Diego Zoo elephant cancers would be informative. The true elephant cancer mortality rate may be higher than our estimates from 644 elephant death reports, partially due to nulliparity, but it remains clear that elephants do not develop 100X more cancer than humans and that Peto's Paradox remains a real and important problem to answer."**

Dr. Rally expresses concerns about Table 1 that contains elephant deaths binned by age, and that this table includes preponderance of younger aged elephants. In response to JAMA peer-reviewers and JAMA editors, we intentionally showed the different age groups of elephant deaths. We also accounted for this issue of different elephant ages as detailed in the supplemental section through the comparison of age-adjusted vs. unadjusted lifetime cancer risk. As explained in the text and supported by previous publications, based on their size and longevity, elephants are calculated to have 100x the lifetime risk of human cancer (Caulin AF, Maley CC. Peto's Paradox: evolution's prescription for cancer prevention. *Trends Ecol Evol.* 2011;26(4):175-182; Roche B, Hochberg ME, Caulin AF, et al. Natural resistance to cancers: a Darwinian hypothesis to explain Peto's paradox. *BMC Cancer.* 2012 Sep 3;12:387; Caulin AF, Graham TA, Wang LS, et al. Solutions to Peto's paradox revealed by mathematical modelling and cross-species cancer gene analysis. *Philos Trans R Soc Lond B Biol Sci.* 2015;370(1673):370.). Moreover, elephants are born at 200-300 pounds and grow 3 pounds per day in less than 10 years to reach over 10,000 pounds at reproductive age, accumulating cellular mass at a much faster rate than humans. With such rapid and massive growth, young elephants are in the age range that should be at very high risk for cancer development. An increase in cancer is clearly not seen in our dataset for either young or older elephants. As explained in our paper, we intentionally analyzed each age group separately and still did not see an increase in cancer deaths specifically to address the concern about including young elephants. We state several times throughout the text that this data needs to be interpreted with caution, but that based on currently available data, there is no evidence to suggest that 100% of elephants are developing cancer as would be mathematically predicted. Our data strongly support what previously has been described as Peto's Paradox (the overwhelming lack of expected cancer deaths in elephants). The fact that we do not see any increase in cancer deaths in the youngest elephants undergoing the highest amount of cell division and increasing cellular mass underscores the fact that elephants must somehow be protected from cancer development.

Dr. Rally describes her concern in her letter about the use of the Elephant Encyclopedia online database (accessed on November 2012 to download data about reported elephant deaths from 1995 to 2012). The use of this lay database was also clearly addressed in the study text, supplement, and recent reply to the letter-to-the-editor. In each of these formats, we readily acknowledge the need for additional databases curated from other sources. However, we disagree strongly with Dr. Rally's characterization of this database as no better than Wikipedia and that it is scientifically invalid. Although it is a lay database, it is the only comprehensive database of its kind and is frequently referenced by elephant veterinarians, elephant scientists, and elephant conservationists. We openly describe this website as a "lay database" in our methods section and discuss that it contains the most information presently available while very clearly addressing its limitations of being publicly curated. As we describe in our paper, we would still expect to see a preponderance of reported cancer deaths in elephants if there was no genetic resistance to cancer in these very large and very long-lived animals. In fact, as explained above, our calculations from this lay database were extremely conservative with an intentional over-estimate of cancer death rates in elephants (because of the stated database limitations) and we still did not find the expected increase in reported elephant deaths due to cancer.

Dr. Rally next writes about our use of necropsy data from the San Diego Zoo: *"There were apparently no efforts made by the authors to control for these confounding variables. Nowhere within the publication is there evidence that the authors used specific exclusion criteria, such as age at death, when selecting necropsies to include in their data set… Equally concerning is the fact that there is no information on the exact number of necropsies included on a per-species basis, except for a mention that there was a 'minimum of 10.'"* We kindly refer Dr. Rally to Table e1 in the electronic supplement **(eTable 1. Tumor Incidence, Mass, Lifespan, and Metabolic Rate of Zoo Mammals)** where we include this exact information that she wants to see, including the exact number of necropsies on a per species basis. In addition, the electronic supplement provides the detailed methodology that addresses her concerns about our analysis. We also address Dr. Rally's worries about using captive animal data in our response to the JAMA letter-to-the-editor, which in fact was written by the members of the San Diego Zoo who curate the very same necropsy data. Also, Dr. Rally writes that *"Comparing data for only 10 individuals of a species who have died after living an artificial life in captivity—where a number of unnatural factors influence their overall health and longevity—cannot be considered an adequate sample size or lead to a valid finding,"* with which we entirely agree, and again refer to eTable1, that includes a detailed description of the many different sample sizes with a wide range of 10 to 76 animals per cohort (excluding elephants with the largest sample size of 644 elephants).

Dr. Rally's expresses concern about the inclusion of two employees of Ringling Bros. Center for Elephant Conservation as co-authors; in fact, both of these Ringling Bros. individuals have PhDs, including a well-respected elephant veterinarian and an elephant reproduction scientist, and both of these individuals contributed to the scientific design and study write up. We declared the grant funding support by Ringling Bros., along with 12 other additional sources of extramural and intramural support for this scientific investigation. All of our research support is clearly listed in the acknowledgement section for the readers of JAMA to see, along with each co-author's affiliation. We can only address

the scientific concerns expressed by Dr. Rally, and so we cannot respond to her concern about how the circus publicizes the scientific findings of this study.

To succinctly summarize our responses to Dr. Rally's letter about the scientific validity of our findings:

1. Dr. Rally seems concerned with 2 main issues: the quality of the elephant necropsy data and the way our results are being used. We have little control over how our results are being used, and that is not a basis for a retraction.
2. Whether elephants get more or less cancer than humans has no bearing on the ethics of the treatment of captive animals. All of the co-authors share Dr. Rally's beliefs on the importance of the ethical treatment of animals.
3. Our conclusions are based on the best data that is currently available, and we never claimed that elephants do not get cancer (only less cancer than expected).
4. While the available data is not perfect, we based our analyses on the necropsies that detailed the cause of death and then presented extrapolations to the necropsies that lacked those details.
5. We were clear that our data comes from captive animals. We agree that disease rates likely differ for animals in the wild, but unfortunately those data are not available. Cancer rates in the wild remain open questions for almost all species. However, animals in captivity may be better comparisons for humans in our modern environments when considering cancer rates.
6. We did present the total number of necropsies for each data point in the supplemental data (we even accounted for the varying numbers in our weighted regression analyses).
7. We documented all of our data sources so that other scientists, like Dr. Rally, can evaluate, criticize and reanalyze it. Dr. Rally does not present any contradictory evidence and so far, the elephant cancer death rates we found in the large online database are consistent with the high quality pathology data from the San Diego zoo, as discussed in the letter-to-the-editor response.
8. If anything, captivity likely increases cancer rates in animals by preventing deaths due to predation, prevention and treatment of infectious diseases, restriction of exercise, and exposure to unhealthful diets. So, it seems likely that we are actually over-estimating cancer rates compared to wild animals.
9. We included juvenile elephant deaths in our analyses because we are comparing cancer rates in elephants to cancer rates in humans which also include pediatric cancers. In fact, younger elephants might be expected to have higher cancer rates due to their massive cellular growth in a short period of time. Age-adjusted lifetime risks were included in our statistical analyses to account for young elephants.
10. As more data becomes available for cancer rates in both captive and wild animals, future publications should be able to improve our estimations of cancer rates in those populations. We look forward to such scientific progress.

In conclusion, all of Dr. Rally's concerns about the use of captive elephant data are addressed in three JAMA publications (original article, supplement, and letter-to-the-editor). Both the Abstract and Conclusion in the JAMA article directly state that *elephants appear to have lower rates of cancer and call for future investigation.* Because we so clearly discuss the data sources, the appropriate analyses given the specific data sources, and the need for replication by others, it would be entirely inaccurate to characterize our findings of decreased elephant deaths due to cancer as a "misleading conclusion." We do not believe our findings can be considered misleading because they are based on careful analysis of the data, the way we reached these findings is clearly and logically defined, and these findings are in agreement with those observed within the elephant community. We believe all of our scientific findings to be reliable and accurate, and we were always very careful to include qualifiers calling for further research on this topic. We hope that this detailed response has sufficiently addressed all of the issues raised by Dr. Rally on behalf of PETA, and that JAMA agrees that our findings represent an important and scientifically valid area of cancer research.

Please do not hesitate to contact us with further concerns or clarifications. We appreciate the opportunity to offer you this response and defense of our science. In case it is helpful, we also have included the relevant responses to the JAMA reviewers about the use of captive elephants.

With warm regards (and on behalf of co-authors),

Joshua D. Schiffman, MD
Edward B. Clark, MD Chair in Pediatric Research
Education Director, Program in Personalized Health
Professor of Pediatrics (Hematology/Oncology)
Adjunct Professor of Oncological Sciences
Investigator, Huntsman Cancer Institute
University of Utah

REVIEWER RESPONSES:

"We agree that it is surprising that there is no obvious increase in cancer deaths with age, which is quite different from human cancer mortality - and may reflect the additional "protective" effects of additional TP53 copies in the elephant. Studies suggest that elephants in captivity do not typically live as long as elephants in the wild (Wiese and Willis 2004), so the captive population we assess here may not fully capture the elderly population of elephants expected to have the highest risk of developing cancer. ***Our estimates of cancer mortality are limited by necessity because the data does not yet exist that would be required for more sophisticated measurements (discussed in more detail below in response to Reviewer #3).*** *As more data is collected, the accuracy of these estimates will further increase and it is possible that we may see more of an age-associated cancer risk. Despite the lack of a large mortality dataset, it is clear that elephants develop less cancer than*

expected. In actuality, the 644 annotated necropsies for elephants is by far (by about an order of magnitude) our best dataset for cancer mortality in a non-human mammal. In order to address these concerns, we have moved this discussion from the supplement to the discussion section of the main text."

"In this study, we use a simplified estimation of cancer mortality rates due to the lack of detailed information in elephant populations. If the standard age population structure of elephants was better understood along with well-documented cancer mortality rates per year (requiring a much larger sample size), the estimates we include would be more comparable to those calculated for humans by GLOBOCAN and the American Cancer Society (calculated as age-adjusted risk per 100,000 people per year). ***Recognizing this limitation and to make our estimates as realistic as possible given the available data for elephants, we calculated inferred mortality rates to account for the unknown causes of death as described in response to Reviewer #2's comments on this issue. Additionally, we calculated the age-adjusted lifetime risk by weighting each age-specific cancer death rate by the proportion of the population in the given age group and summing these for the cumulative risk.*** *This is based on the methods used for human data; however, there is not a standard population structure to use for elephants so we have instead applied the distribution of ages we find in this population of 644 elephant deaths. Reassuringly, the age-adjusted lifetime risk is not statistically different from the crude calculation of lifetime risk of cancer mortality in elephants (4.81% vs 4.82%). As more data becomes available these cancer mortality rates can be refined to enable a more direct comparison with human cancer mortality rates. We have added text to both the discussion section of the manuscript and the supplemental methods to address these caveats."*

"We agree with the reviewer that it would be helpful to generalize these cancer mortality estimates to a more general population; however we have not been able to find and obtain the necessary information outside of captive elephants to make those calculations. The major piece of information on which the human data relies is the standardized population data that provides information on the distribution of each age group. ***When we used the distribution of ages in the population of dead elephants that we collected, this did not affect our results as described in the previous response.*** *We have added text to both the discussion section in the main text as well as the supplemental content to address these limitations and to provide more background on our calculations."*