NOVEMBER 2021

Modernizing Cancer Research

People for the Ethical Treatment of Animals

Executive Summary

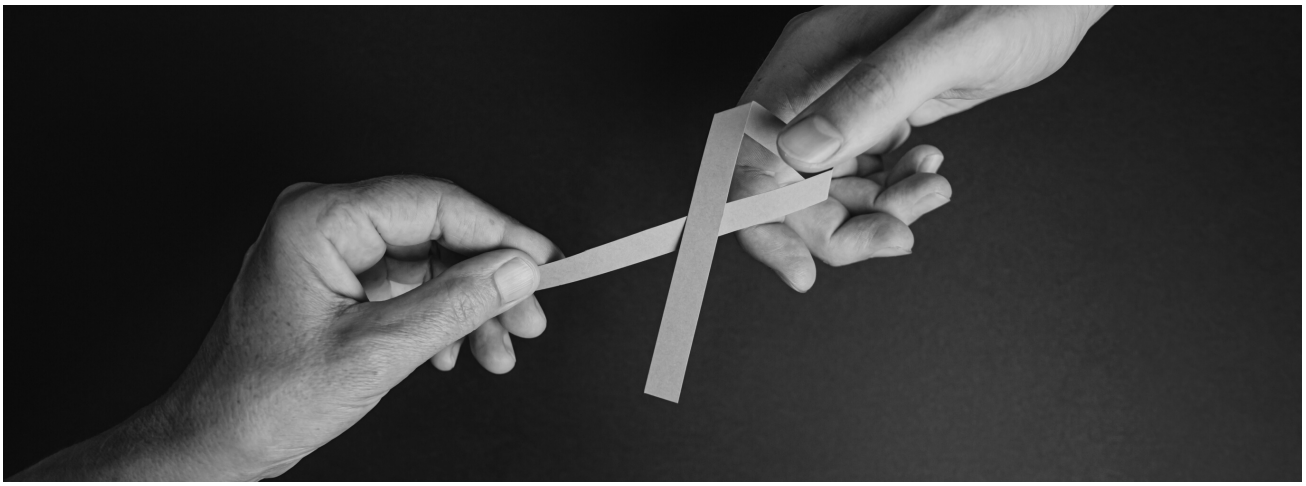
This year marks the 50th anniversary of former President Richard Nixon's "war on cancer," yet cancer has remained the second leading cause of death in the U.S. The 27% decrease in cancer rates over the past two decades is attributable primarily to personal preventive measures, including not smoking, eating more fruits and vegetables, and going in for screening tests—not to the results of biomedical research. This lack of progress in treating and curing cancer is attributable, in large part, to a misplaced reliance on animal tests, even though the results of these tests do not reliably save human lives.

Fortunately, progress has been made in the development of sophisticated test methods based on human biology (i.e., non-animal methods). These reliable, non-animal test systems show genuine promise and should be used for developing and assessing treatments for cancer in humans. Additionally, protection from potentially carcinogenic chemicals can be achieved using non-animal tests to identify them so that we can limit or avoid exposure. We will provide information about these methods and how they can be implemented to save lives. We also recommend the following actions, which are essential to accelerating the development of life-saving research efforts:

- Reallocating National Institutes of Health intramural and extramural research funding toward animal-free, human-relevant models
- Commissioning an unbiased, multi-stakeholder committee in order to systematically review the translatability of cancer research and carcinogenicity assessment in animals to human patients
- Providing regulators and researchers with opportunities to receive free training and information about the use of human-relevant models
- Adopting legislation to replace archaic requirements of lifetime tests on rats and mice for carcinogenicity assessment with rapid, reliable, and human-relevant models
- Increase the amount of federal funding allocated to cancer prevention.

Prepared By

Emily Trunnell, Ph.D.
Gina Hilton, Ph.D.
Katherine Roe, Ph.D.



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I. Introduction

In 2019 and 2020, cancer was the second leading cause of death in the U.S., with officials estimating that close to 600,000 American died from cancer in 2020 [1,2]. Approximately 39.2% of people in the U.S. can expect to be diagnosed with cancer at some point during their lifetime, and despite significant investment in research for cancer therapies, only 67.7% of these individuals will survive for longer than five years following their diagnosis (Figure 1) [3]. In addition, certain population groups are disproportionately affected. For example, in the U.S., African Americans are 25% more likely to die of cancer than Caucasians [4].

Figure 1: "Based on data from SEER 18 2011–2017. Gray figures represent those who have died from cancer of any site. Green figures represent those who have survived 5 years or more" [3].

The proportions of lifestyle factors that influence the development of cancer, leading to a reduction in rates [5], have been estimated as 30% to 35% from diet, 25% to 30% from tobacco, 15% to 20% from infections, and 4% to 6% from alcohol (Figure 2) [6]. For example, consumption of red and processed meat has been identified as an increased risk factor for colon cancer [7]. On the other hand, healthy plant-based diets high in fruits, vegetables, cereals, nuts, seeds, legumes, and vegetable oils are rich in bioactive compounds (i.e., fiber) that have anti-inflammatory, anti-oxidative, and anti-carcinogenic properties—thus reducing the risk of developing cancer [8].

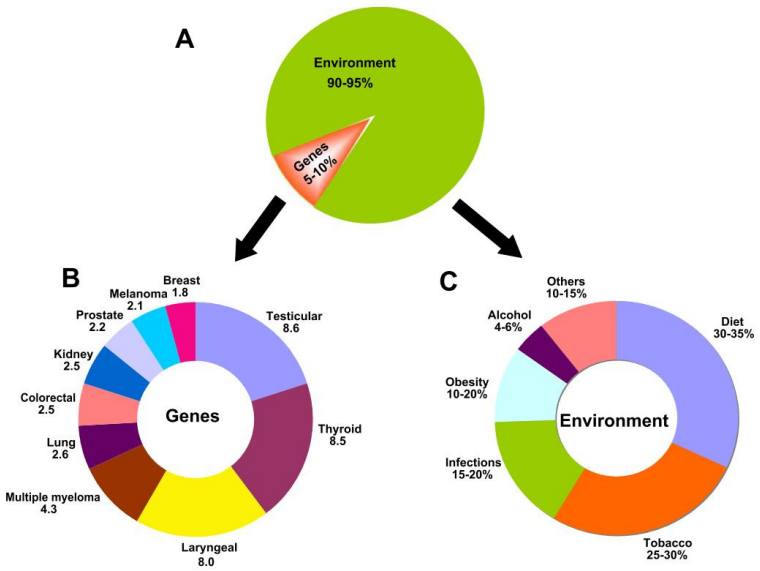


Figure 2. "The role of genes and environment in the development of cancer. A The percentage contribution of genetic and environmental factors to cancer. The contribution of genetic factors and environmental factors towards cancer risk is 5–10% and 90–95% respectively. B Family risk ratios for selected cancers. The numbers represent familial risk ratios, defined as the risk to a given type of relative of an affected individual divided by the population prevalence. The data shown here is taken from a study conducted in Utah to determine the frequency of cancer in the first-degree relatives (parents + siblings + offspring). The familial risk ratios were assessed as the ratio of the observed number of cancer cases among the first degree relatives divided by the expected number derived from the control relatives, based on the years of birth (cohort) of the case relatives. In essence, this provides an age-adjusted risk ratio to first-degree relatives of cases compared with the general population. C Percentage contribution of each environmental factor. The percentages represented here indicate the attributable-fraction of cancer deaths due to the specified environmental risk factor" [6].

Because of the impact of lifestyle changes and regular screening tests [6] on preventing cancer, legitimate questions are being raised about both the billions of dollars beings invested in cancer research and the reasons why cancer still claims so many lives every year. This white paper will highlight the advantages and limitations of animal and non-animal approaches to cancer research and carcinogenicity testing and will supply figures related to federal funding of cancer research. It will also outline a plan for shifting the focus of cancer research to the use of animal-free, human-relevant methods, which would ensure greater progress toward eliminating cancer over the next 50 years.

II. History

In 1937, the National Cancer Institute (NCI) was established in response to the ongoing increase in cancer-related mortality in the U.S. [9]. Following decades of rising cancer rates, in 1971, the National Cancer Act, which allocated $100 million to the NCI to further enhance research efforts to find a cure for cancer, was signed into law by President Nixon [10]. At the time, cancer was the second leading cause of death in the country, as it has typically remained to the present day, surpassed only by heart disease and, briefly, by COVID-19.

The first order of business of the “war on cancer” was to amend the Public Health Service Act of 1944 to give the NCI director the ability to plan and develop a National Cancer Program. It also initiated the NCI Professional Judgment Authority, which creates a direct channel from the NCI director to the president and Congress for establishing the annual NCI budget [11]. The newly amended act also created a National Cancer Advisory Board to aid the NCI in developing programs, cancer centers, and training initiatives for physicians and researchers. The “war on cancer” made funding available for the NCI to establish 15 cancer research centers as well as an international cancer data bank [10].

III. Federal Spending on Cancer Research and Prevention

Since the introduction of the National Cancer Act, more than $140 billion have been spent on cancer-related initiatives [12]. Nevertheless, for the last 50 years, cancer has remained the second leading cause of death in the U.S. One major roadblock to meaningful progress to cure cancer has been the continued misguided allocation of resources toward research using rats, mice, dogs, nonhuman primates, and other animals, even though this testing on animals has never been formally validated to determine whether the results are relevant to humans [13]. We are losing the "war on cancer," in part because of this misallocation of funding.

Although there has been a substantial reduction in cancer rates attributed to personal prevention, only a small proportion of NCI funding has been allocated for cancer prevention. For example, in 2019 less than 6% of funding was spent on cancer prevention and control whereas almost 70% of funds were dedicated to research [14]. The percentage of NCI’s funds dedicated to cancer prevention and control has stayed relatively stable over the past several years, despite increases in overall budget (Figure 3) [15].

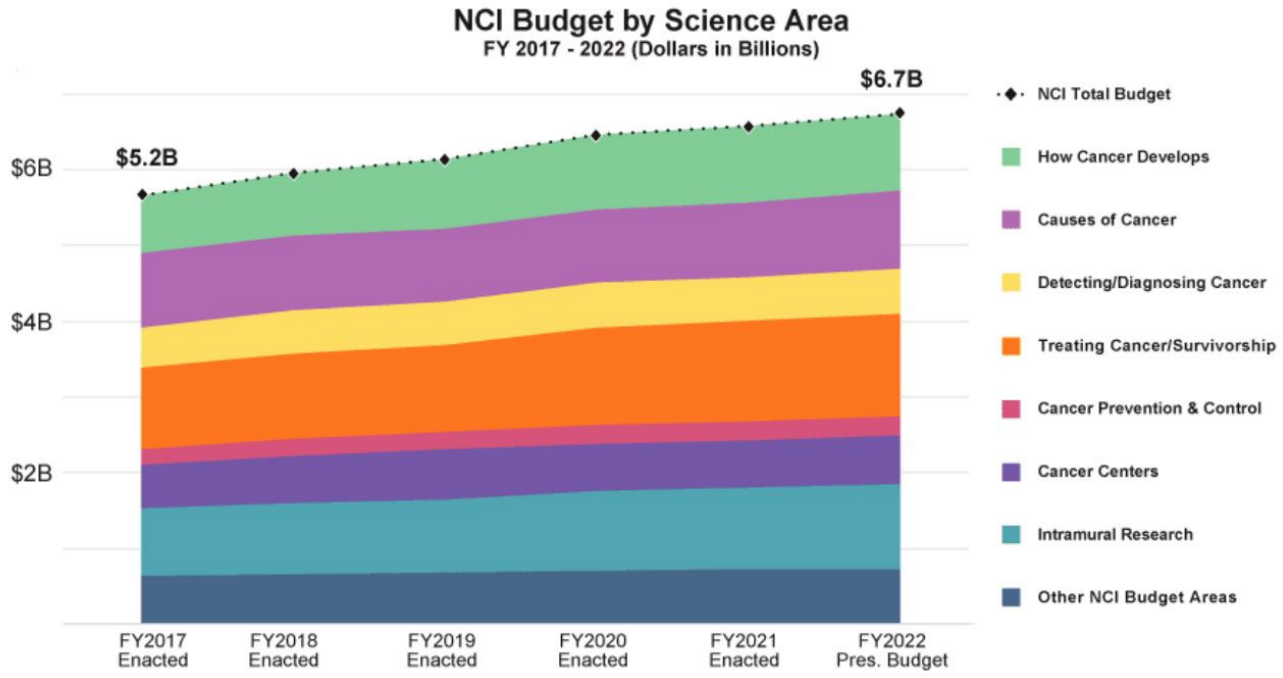


Figure 3. NCI budget by science area from 2017 to 2022 [15].

Although significant funding is allocated to research, close to 600,000 people died of cancer in the U.S. in 2020 [2], which indicates a clear misstep in the current research approach. To wage an effective "war on cancer," research funding needs to be reallocated to cutting-edge tools based on human biology that have a far greater potential for generating treatments and cures for cancer in humans.

IV. Cancer Research

A. Methods of Animal Cancer Experimentation and Their Limitations

There are several methods by which rodents—predominantly mice—are used in basic and translational cancer experimentation, the most common being xenotransplantation and genetic engineering. Although mice are by far the most common animals used, other animals—including rats, zebrafish, dogs, nonhuman primates, tree shrews, and pigs—have also been used for cancer experimentation [16].

1. Xenograft Models

In xenograft models, human or animal cancer cells are transplanted either under the skin or into an organ of immunocompromised rodents, who may then be treated with a chemical or test substance of interest [17]. Xenograft models may be preferred by some experimenters because of their ease of use and low cost compared to genetically engineered mice, in addition to the longer history of their use. However, there have been many documented concerns with xenograft models.

One of the major disadvantages of xenograft rodent cancer models is the limited application of knowledge gained from these experiments to treat cancer in humans. For example, researchers use aggressive cancer subtypes in xenograft models, which creates a study bias against less aggressive tumors. They also implant tumors under the skin in mice, which does not recapitulate the various sites of tumor formation in human patients. Furthermore, xenograft tumors are often superficially vascularized (meaning that they lack the blood flow of naturally occurring tumors) and there is a lack of interaction between the tumor and the supportive connective tissues or blood vessels [17,18]. Another major limitation of xenograft cancer models is that the mice and rats used have compromised immune systems, so the tumors they exhibit do not represent the behavior of naturally occurring cancer in most humans [17]. This also means that immunomodulatory agents can't be reliably tested using xenografted animals [19]. Additionally, two common host mouse strains in xenograft studies, severe combined immunodeficient and nude mice, have defective DNA repair and overall frailty, respectively, that limit which agents can be tested on these animals [19].

NCI Director Norman E. Sharpless and former MD Anderson Cancer Center President Ronal DePinho noted that a significant drawback of xenograft models is "the fact that these systems model cancer as if it was a disease of homogeneous rogue cells," when clinically, "[c]ancers are better likened to a complex organ system with distinct and heterogeneous neoplastic and host components acting in concert to maintain the tumour" [19]. The pair also noted that another problem with xenograft analyses is that "many agents that show consistent and potent anticancer activity in specific xenograft models prove to be of limited use in the therapy of human cancer. This single fact is a major contributor to the low success rate of novel therapeutics when first tested in humans" [19].

Researchers are beginning to understand why so many drugs that look promising in xenograft experiments end up failing in human trials. Following an analysis of 1,110 mouse xenograft tissue samples from 24 different cancer types, Ben-David *et al*. reached a conclusion that challenged the ability of xenograft models to predict patients' responses to therapy. They found that transplanting human cancer cells into mice altered the genetic composition of their cells in ways unlikely to occur in humans, which, in turn, altered the responses that the cells had to chemotherapy drugs [20]. Essentially, when human tumor cells are transplanted into mice, they develop characteristics of mouse cells, which are not relevant to human biology.

2. Genetically Engineered Cancer Models

Experimenters create genetically modified (transgenic) mice by inducing the expression of oncogenes or by inactivating tumor-suppressing genes [21]. However, with these methods, researchers are often unable to control the level and pattern of the gene expression or gene inactivation, thus failing to mimic the sporadic and multistep nature of tumor growth seen in natural tumor development [21]. In addition, random integration of the oncogenes can result in unexpected outcomes that would not be present in human patients [21]. These models are also time-consuming, are costly to create, and require large numbers of animals because of extensive breeding requirements [22,23].

3. Environmentally Induced Cancer Models

Environmentally induced cancer models involve exposing animals to known cancer-causing agents, such as asbestos, radiation, or some viruses and/or bacteria [17,24]. According to Kristopher K. Frese and David A. Tuveson, "[S]uch models develop a restricted subset of tumour types and grades with incomplete penetrance and variable latency" [24]. These are less often used than xenografts or genetically engineered animals.

4. 'Humanized Mice'

Because the makeup and function of the immune system varies significantly between humans and mice, some researchers hope "humanized mice" will serve as a missing translational link between the two species. Experimenters create these mice by irradiating them to destroy their immune system and attempting to repopulate it with human immune cells [25]. This approach has various disadvantages, many of which resemble the issues described for xenotransplantation models: Primarily, the animal's immune system has been completely disrupted and is in no way relevant to human physiology. Attempts to reconstitute the immune system may be incomplete, and the animal's native immune system attempts to survive, meaning that in these models, two different immune systems typically coexist in one animal [25]. In a report on the need for more human-relevant models for immuno-oncology research, the European Commission's Joint Research Centre (JRC) noted that even if humanized mouse models were to be improved, they would still be lacking because of the "sub-optimal development of specific human immune cell types … or the residual mouse immune components" [26].

B. Failures of Translation

Oncology drugs have a low likelihood of approval, with success rates estimated at just over 3% (Figure 4) [27,28]. Although study design and other logistical issues can be problematic, fundamental biological differences between humans and other animals most severely limit the translation of animal model findings to the clinic. Mak *et al*. highlighted this crucial point in their 2014 review of failed translation of animal models in cancer research: "[M]ost futilities in fact originate from molecular mechanisms of the drug(s) tested. . . . Crucial genetic, molecular, immunologic and cellular differences between humans and mice prevent animal models from serving as effective means to seek for a cancer cure" [29].

In his 2010 article "TGN1412: From Discovery to Disaster," Husain Attarwala recounted the tragic outcome of the 2006 clinical trial for Theralizumab (TGN1412), an immunomodulatory drug developed for the treatment of immunological diseases such as multiple sclerosis, rheumatoid arthritis, and certain types of cancer. He wrote, "After [the] very first infusion of a dose 500 times smaller than that found safe in animal studies, all six human volunteers faced life-threatening conditions involving multiorgan failure for which they were moved to [the] intensive care unit" [30].

	All oncology
Phase 1 to Phase 2	65.0 percent
Phase 2 to Phase 3	38.0 percent
Phase 3 to approval	24.1 percent
Phase 1 to approval*	3.3 percent

Figure 4. "Path-by-Path Estimates of Probability of Success (PoS) of Oncology and Orphan-Oncology Trials" [28].

Five of the six participants had to remain hospitalized for three months after the initial dose, while the other was comatose. Even six months later, participants suffered from headaches and memory loss. One had to have toes and fingers amputated as a result of gangrene [31]. Studying this and other trials, Attarwala concluded, "Drugs showing safety and efficacy in preclinical animal models may show very different pharmacological properties when administered to humans" [30].

"The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades—and it simply didn't work in humans." - Richard Klausner, MD; National Cancer Institute Director, 1995-2001

Mak *et al.*, along with Sharpless and DePinho, describe additional cancer clinical trial disappointments, including the failures of thiazolidinediones, angiogenesis inhibitors, IPI-926, matrix metalloproteinase inhibitors, farnesyltransferase inhibitors, and efforts to create cancer vaccines, all of which were first tested and found to be successful in animal experiments using cancer models [19,29].

The European Commission's JRC wrote, "Recent advances in immuno-oncology research highlight the limitations of commonly used animal models in developing new approaches for cancer therapy. These models have failed to recapitulate the variable responses and potential toxicity seen in clinical settings" [26].

In addition, former NCI Director Dr. Richard Klausner stated, "The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades—and it simply didn't work in humans" [32].

C. Welfare Concerns

A significant welfare issue is that mice and other animals used in cancer experimentation experience pain and distress. Animals may be made to grow tumors in sensitive areas such as their footpads, tails, eyes, bone, or muscle and may not be given analgesia for fear that it could interfere with scientific results (even though human patients would likely be given this type of care) [33,34]. Anecdotally, journal editors and reviewers have documented issues with experimenters who have allowed animals to endure tumor burdens exceeding what is typically approved by oversight committees. One such example of a paper published in *Nature* involved mice who were made to have tumors that grew to 7 cubic centimeters in size—more than half the size of most mice (the permitted size was 1.5 cubic centimeters). Discovery of this error led to a correction of the paper, which was later retracted by the journal [35]. Figure 5 shows an image of a nude mouse used in cancer experimentation.

Figure 5. Image from a PETA investigation, courtesy of PETA.

Genetically modifying mice introduces welfare concerns that may be unanticipated by experimenters and animal care support staff. The location of genetic integration often cannot be controlled, resulting in a high potential for off-target effects, unexpected sites of tumor development, and even lethality [23,33]. At early stages, the creation of these models may include invasive procedures, such as induction of superovulation in females, sperm collection from males, artificial insemination, implantation of embryos, and tissue sampling for genotyping [23]. In addition, a large number of animals may be required for breeding purposes, and "surplus" animals who do not express the genetic modification of interest are often considered expendable and are consequently euthanized [23].

D. Animal-Free Research Methods

Given the many shortcomings of cancer modeling in animals as well as the astonishingly low translational success rate of such models, it's clear that they are not good models for human cancer research. In light of this and the pain and suffering experienced by the animals who are used, it should be a priority to move away from animal models and focus instead on human-relevant methods. In August 2021, the JRC report on immuno-oncology highlighted important publications that describe promising advanced, non-animal models. These studies employed human-based, non-animal methods for developing immunotherapies, studying cancer initiation and development, exploring anti-cancer therapies, studying immunomodulation of cancer physiology or potentially effective strategies for enhancing the anti-tumor immune response, determining molecular features that can represent biomarkers in specific cancer pathogenesis, exploring adoptive cell therapies and virotherapies, and more [26]. A grouping of those uses into more general applications can be seen in Figure 6.

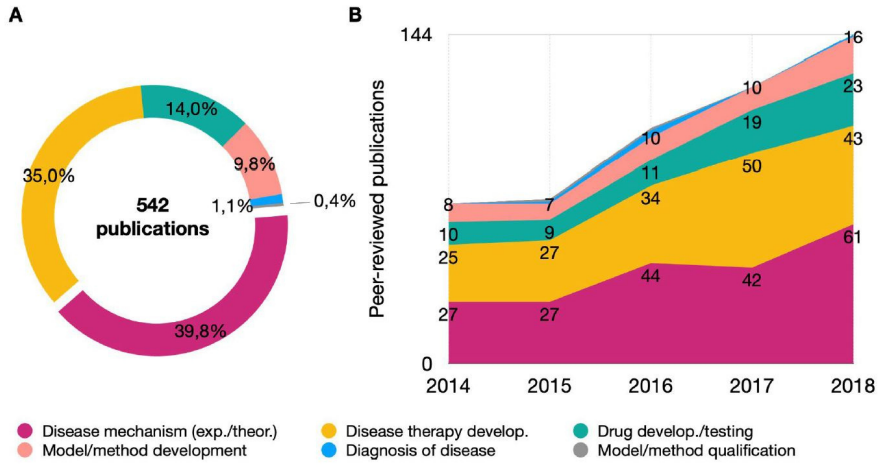


Figure 6. "Six main applications for non-animal models in immuno-oncology research were identified. Panel A shows the percentage of each reported application in all retrieved articles, including publications from 2019. Panel B shows the distribution of articles by non-animal model application from January 2014 to December 2018 (2019 data are not included since only 3 months were analysed). The number of articles per year for the four major applications (disease mechanism, disease therapy development, drug development/testing, model/method development) are shown" [26].

The following are some examples of non-animal methods currently being used for cancer research:

1. Three-Dimensional Printing

- The prestigious Institution of Engineering and Technology (IET) global Harvey Engineering Research Prize was recently awarded to Portuguese scientist Rui L. Reis for his work using tissue engineering to create reliable three-dimensional (3-D) engineered functional cancer disease models. According to IET, his innovative research will "help to predict the efficacy of novel cancer drugs and potential therapies, avoiding a range of unnecessary animal tests, and preclinical and clinical trials of doomed-to-fail new drugs" [36].
- At the University of Stuttgart, scientists are working on 3-D printed tissue platforms that can be assembled into realistic tumor models and used for testing cancer drugs [37].
- Three-dimensional printing is being used to produce precise replicas of tumors using patients' own cells in the bioink [38].
- Vascular tumor models have been created using 3-D bio-printing to mimic key steps of cancer metastases. These tissues can be used to explore the molecular mechanisms of tumor progression and metastasis, identify therapeutic agents, and screen anticancer drugs [39].

2. *In Vitro* Experiments

Examples of recent, human-relevant in vitro cancer research include the following:

- The study of patient-derived human brain organoids to develop personalized therapies for deadly glioblastomas (Figure 7) [40, 49]
- The development of a human blood vessel–on-a-chip to advance new cancer therapies that may inhibit new blood vessel formation to slow tumor growth [41]
- The use of a tumor microenvironment–on-a-chip to create precision medicine tailored to individual patients and specific cancer types (Figure 8) [42]

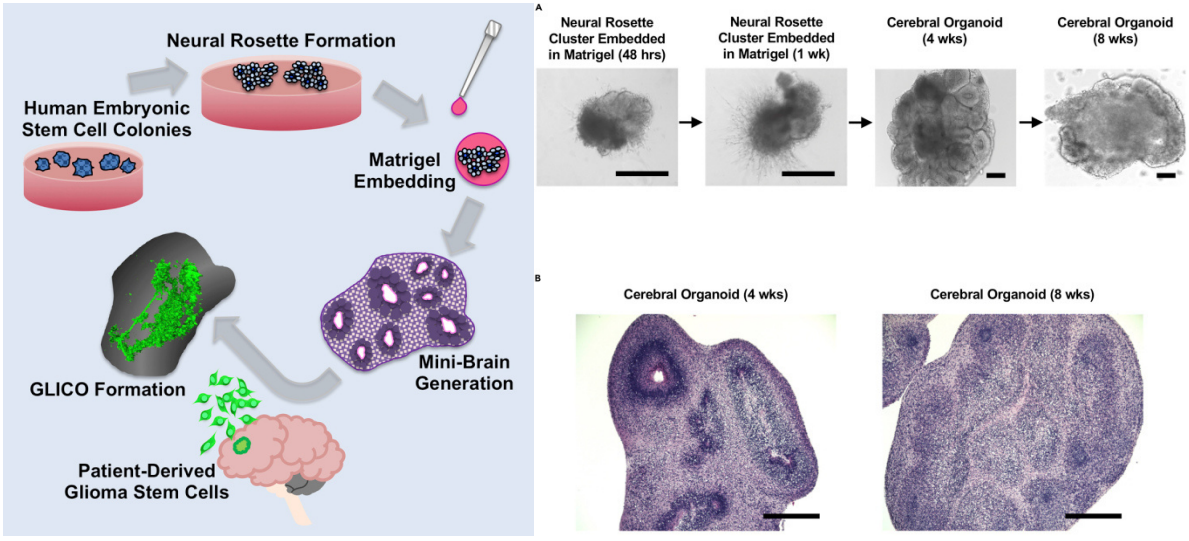


Figure 7. Graphical abstract and figure from "Generating Patient-Derived Gliomas Within Cerebral Organoids" by Amanda Linkous and Howard A. Fine. The authors describe the right panel as follows: "Stage-Specific Morphology of Cerebral Organoids. (A) Bright-field microscopy of cerebral organoid maturation. (B) Hematoxylin and Eosin staining of cerebral organoids at 4 weeks and 8 weeks post-Matrigel embedding; scale bars, 400 μm" [49].

- A second-generation lung-on-a-chip with stretchable alveoli made from a biological membrane developed by University of Bern scientists (Cells from cancer patients undergoing lung resections were cultured on these chips to provide a predictive tool for drug screening and precision medicine [43])

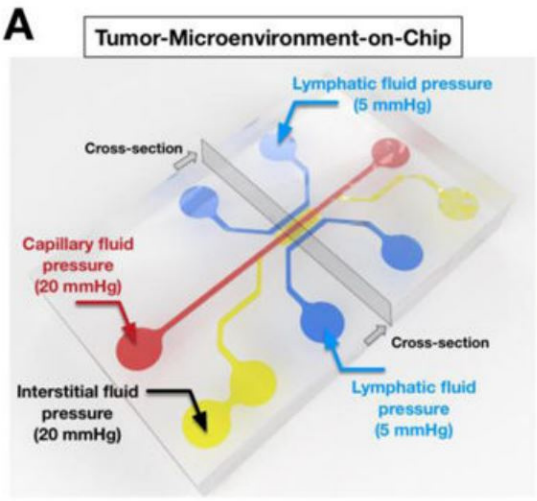
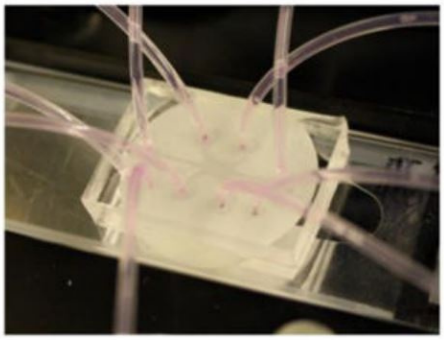


Figure 8. "Schematic of the fabricated T-MOC [tumor microenvironment on a chip] platform and its operating pressure conditions" [42].



- A SUM Breast Cancer Cell Line Knowledge Database, created by scientists at the Medical University of South Carolina, which allows researchers to gain a better understanding of 40 different breast cancer cell lines and speed up the development of new gene-targeted therapies [44]
- The use of CRISPR/Cas9 to tag and track single telomerase enzymes in a cell, allowing researchers to begin to understand how cancer-associated mutations drive defects in telomere homeostasis [45]
- Single-cell analysis of mammary tumor organoids, conducted by scientists at Harvard Medical School, which found that the pattern of protein expression matched that of the original tumor, further validating this model [46]
- Cancer-on-a-chip models to help understand the tumor microenvironment and its role in metastasis (Scientists believe that these models can "drastically change the way we can test drug efficacy, or even develop new therapies to specifically prevent metastasis" (Figure 9) [47])
- The use of a human body-on-a-chip device (Figure 10). (Researchers from Hesperos, Inc., circulated blood-like fluid through a multichamber chip cultured with various tissue types and cancer cells, along with anti-cancer drugs, to assess efficacy and safety simultaneously. The system correctly identified known adverse effects and could assess the efficacy of different drugs and drug combinations [48])

In addition, the JRC's report noted the many advantages of tumor-on-chip (ToC) models, which are generated by "co-culturing tumor and stroma cells (immune cells, endothelial cells, fibroblasts) within 3D biomimetic matrices in microfluidics devices" [26]. According to the JRC, "They are immunocompetent, in that they recapitulate the interplay between immune and cancer cells. They can be personalised by introducing patient-derived autologous primary cells, and they can be treated with drugs and visualised in real time by video microscopy. ToC is a disruptive approach to investigate the drug-dependent plasticity of tumor ecosystems and the mechanisms underlying immunotherapy resistance" [26].

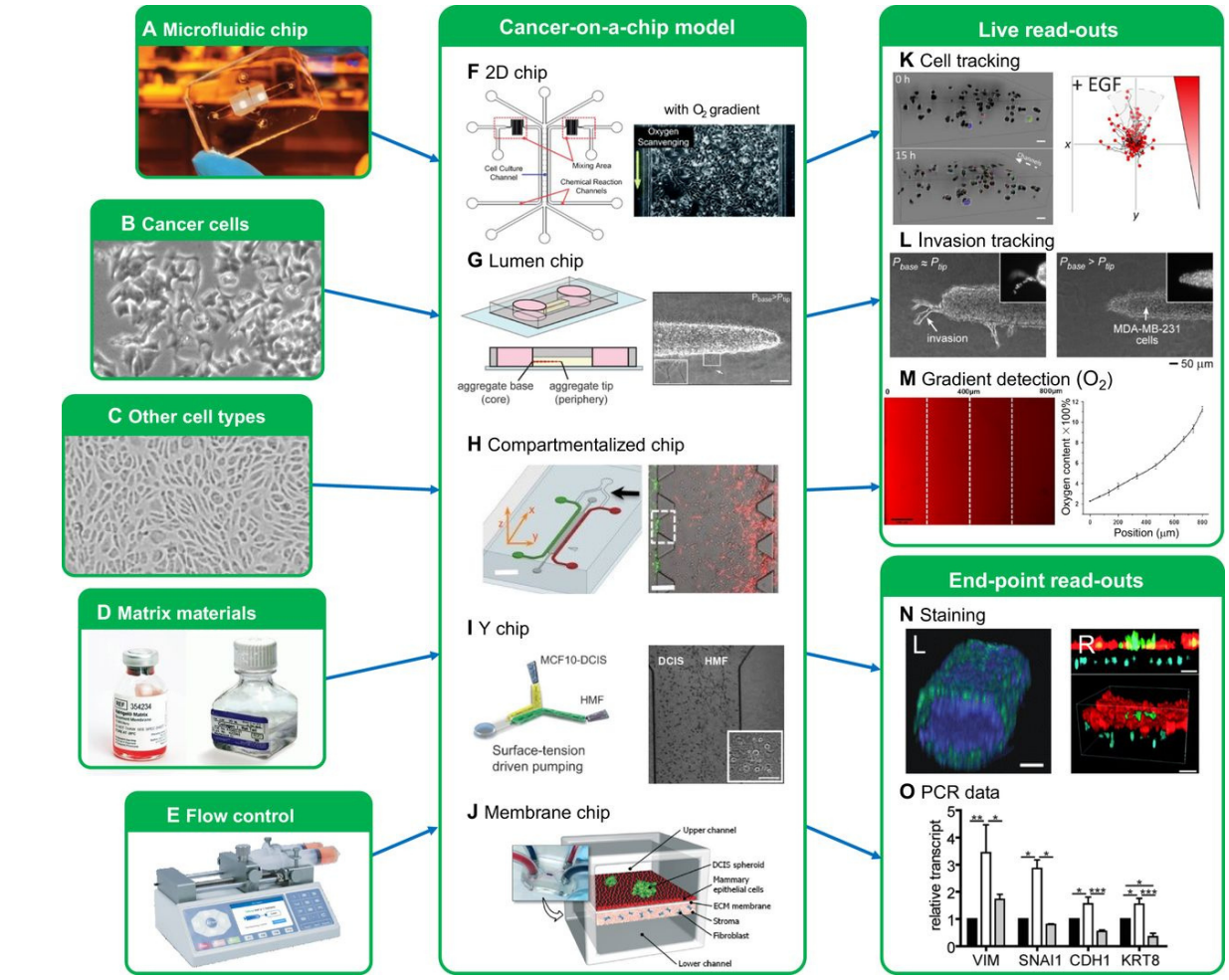


Figure 9. "The key input elements of CoC [cancer-on-a-chip] models are: (A) a microfluidic chip, (B) cancer cells, (C) additional cells (optional), (D) matrix materials (optional) and (E) equipment to control fluid flow, such as a syringe pump. Using these elements, the different CoC model types can be built" [47]

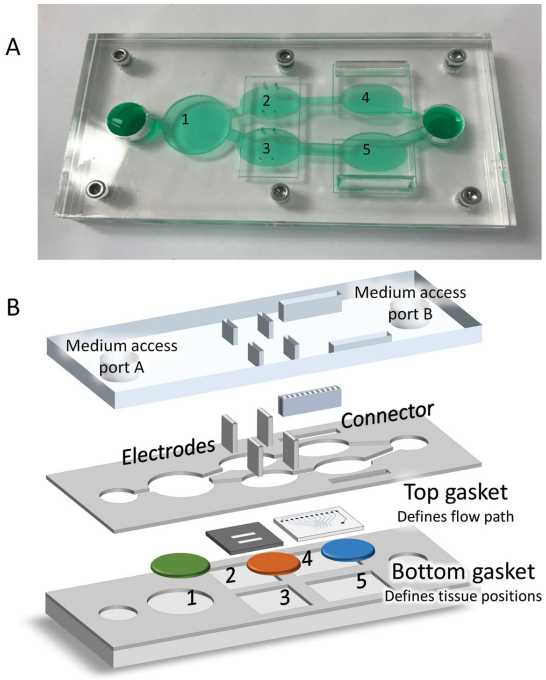


Figure 10. "Five-chamber reconfigurable multi-organ system. (A) Photograph of the multi-organ system filled with green-colored dye for visualization. Scale bar, 2 cm. (B) Exploded view schematic representation of the platform assembly and design used in the system 2 study of tamoxifen. Chamber 1 houses hepatocytes on coverslips. Chambers 2 and 4 are cardiac cantilevers and microelectrode arrays (MEAs), respectively. Chambers 3 and 5 are for cancer cells SW-962 and MCF-7. Drugs were applied to medium access port A and initially passed over the liver to mimic aspects of first pass metabolism" [48].

3. Human Genomics

- An integrated analysis of 2,658 whole-cancer genomes and matching tissues from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium and The Cancer Genome Atlas reported groundbreaking results describing noncoding mutations driving cancer, new mutational signatures, and an evaluation of a range of specialized features of cancer genomes (Figure 11) [50].
- A recent study conducted at the University of California–San Diego may have found a reason why humans are more prone to develop carcinomas, even compared to our closest evolutionary nonhuman relatives, chimpanzees: the uniquely human gene SIGLEC12.51 Siglec-XII, the protein produced by SIGLEC12, is present in a high proportion of advanced carcinomas in humans [51].
- By sequencing DNA and RNA in human skin cells, researchers at the University of California–San Francisco have analyzed which signaling pathways are disrupted in the evolution of human melanoma [52].

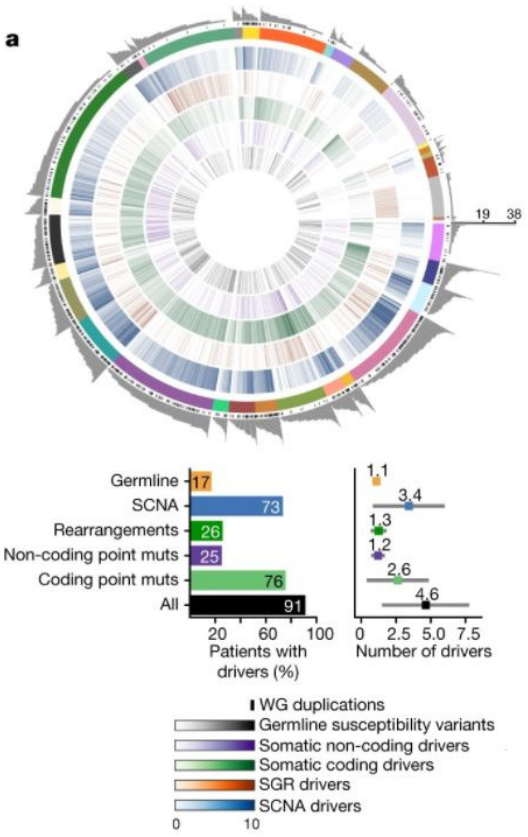


Figure 11. "Top, putative driver mutations in PCAWG, represented as a circos plot. Each sector represents a tumour in the cohort. From the periphery to the centre of the plot the concentric rings represent: (1) the total number of driver alterations; (2) the presence of whole-genome (WG) duplication; (3) the tumour type; (4) the number of driver CNAs [copy number alterations]; (5) the number of driver genomic rearrangements; (6) driver coding point mutations; (7) driver non-coding point mutations; and (8) pathogenic germline variants. Bottom, snapshots of the panorama of driver mutations. The horizontal bar plot (left) represents the proportion of patients with different types of drivers. The dot plot (right) represents the mean number of each type of driver mutation across tumours with at least one event (the square dot) and the standard deviation (grey whiskers), based on n = 2,583 patients" [50].

4. Computational Biology and Artificial Intelligence

- Researchers have developed a new computational technique called CopyKAT to differentiate accurately between data from cancer cells and the various normal cells found within tumor samples [53] This technology can be used to understand the genomics of malignant cancer cells, among other things.
- In a clinical study, an artificial intelligence algorithm successfully diagnosed prostate cancer in slides of needle biopsies. The algorithm was able to assess both the stage of the disease and other important findings accurately and is already being used in practice (Figure 12) [54].

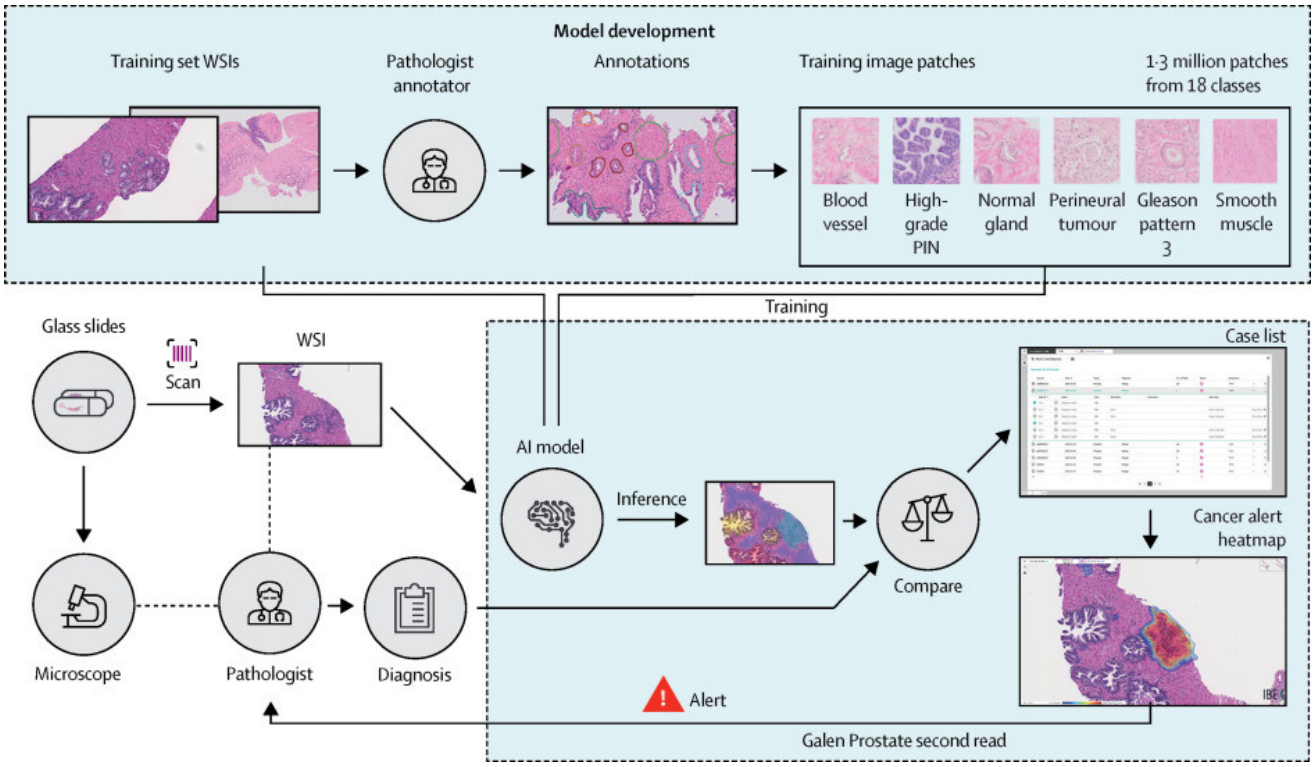


Figure 12. "Overview of the algorithm and clinical deployment of the Galen Prostate second read system. AI=artificial intelligence. WSI=whole image slide. PIN=prostatic intraepithelial neoplasia" [54].

- Scientists and computer programmers are using deep learning to generate profiles of cancer cells in order to identify markers that may be meaningful for drug discovery and predict drug responses for individual patients [55].

5. Bionics

Researchers at the Hebrew University of Jerusalem have now used a bionic chip fitted with microsensors that generate real-time data to shed light on the negative effects of the cancer drug cisplatin and test out an alternative therapy [56].

Importantly, scientists using non-animal methods for cancer research are faced with a smaller translational hurdle, because all human-relevant methods are grounded in human—instead of rodent—biology.

6. Spontaneous Cancer Models

Some researchers now recognize the utility of studying naturally occurring types of cancer in companion animals who are taken to veterinary clinics by their caretakers [17]. According to the American Veterinary Medical Association, one in four dogs will develop a neoplasia at some stage in their life and almost half of dogs over the age of 10 will develop cancer [57]. Compared to studies conducted on animals in laboratories, naturally occurring tumors in companion animals are more similar to naturally occurring tumors in human patients, in that they occur in bodies with intact

immune systems, are heterogeneous, develop recurrent and drug-resistant disease, and metastasize to distant sites [17]. When conducted appropriately, clinical trials with companion animals have the potential to promote developments in animal oncology and could encourage further developments in human oncology as an additional advantage.

V. Carcinogenicity Testing

A. Required Tests and Their Limitations

Rodent cancer bioassays are currently required by regulatory agencies to assess potential carcinogenicity—a chemical's capacity to cause cancer—in agrochemicals, food additives, and pharmaceuticals. These rodent bioassays are conducted as lifetime tests on at least 800 mice and rats for 18 to 24 months to assess the carcinogenicity potential of a single substance [58,59]. Daily chemical dosing begins as soon after weaning as possible, and the chemicals are usually administered orally by gavage (i.e., a needle is passed through the animal's esophagus for direct administration of the substance into the stomach). Alternately, chemicals can be administered by either rubbing the substance on shaved skin or by inhalation. Animals are then observed for clinical signs, such as weight changes or the growth of tumors. Animals who die (or are euthanized) during the study and those killed at the end of the study are necropsied, and signs of tumors are then recorded. These animals endure painful, long-term tumor growth and have been known to suffer from lethargy, nausea, and death.

Decades of research has underscored limitations in these rodent cancer bioassays. Confidence in the tests is low, and regulatory decisions based on animal cancer data are frequently disputed. The cancer bioassays are known to be poorly reproducible [60], animal-intensive, and they lack relevance to human biology [61]. These tests aren't able to keep pace with the need to assess emerging human-related exposure concerns (e.g., environmental pollution) in a health-protective and timely manner [62–65]. Politicians, advisory groups, regulators, industry stakeholders, and health advocates now acknowledge the limitations of the rodent cancer bioassay and agree that there is a critical need for more reliable human-relevant data [65–67]. Because of the limitations of the rodent bioassays, scientists are working to replace rodent cancer tests for both ethical and scientific reasons.

B. Animal-Free Test Methods

Scientific advances and a greater understanding of cancer biology [50,68,69] are shifting regulatory testing into a new paradigm, in which chemical risks to humans can be effectively evaluated by using integrated approaches for carcinogenicity assessment [62–64,70,71]. Consider the following examples:

- Computational models and databases have been developed to predict the chemical potential for carcinogenicity [72], including CASE Ultra, Derek Nexus, Lazar, the OECD QSAR Toolbox, the Leadscope SAR Carcinogenicity Database, the U.S. Food and Drug Administration's Center for Drug Evaluation and Research's rodent carcinogenicity (Q)SAR models, VEGA HUB, and the U.S. Environmental Protection Agency's OncoLogic.

- Tissue engineering and cancer organoids are rapidly emerging to offer human-relevant models to assess therapeutic responses in drug development as well as environmental exposure assessment to inform carcinogenic risk to humans [73,74].
- Epidemiology studies yield population-level evaluations of correlative relationships between chemical exposure and carcinogenic effects, which can be used to inform susceptibility in cancer-risk assessments [75,76].
- Weight of evidence assessments can be used to determine whether integrated information is sufficient to draw a conclusion about the carcinogenic potential of a substance without conducting rodent cancer bioassays [66,67,77,78]. For example, a computational tool called Kaptis is being developed to help support weight of evidence-based chemical risk assessment [79].

To optimize carcinogenicity testing approaches, researchers need funding, human data (e.g., from product development, clinical trials, clinical experience, and epidemiology), and international collaboration to facilitate development and uptake of fit-for-purpose animal-free methods to support human-relevant carcinogenicity testing programs [75,80–85].

VI. Summary and Recommendations

The U.S. is spending an extraordinary sum of money attempting to cure cancer and to test carcinogenicity in animals while discovering very little about therapies or cancer protection for humans.

To protect humans from cancer, the following steps should be taken:

- Reallocating National Institutes of Health intramural and extramural research funding toward animal-free, human-relevant models
- Commissioning an unbiased, multi-stakeholder committee in order to systematically review the translatability of cancer research and carcinogenicity assessment in animals to human patients
- Providing regulators and researchers with opportunities to receive free training and information about the use of human-relevant models
- Adopting legislation to replace archaic requirements of lifetime tests on rats and mice for carcinogenicity assessment with rapid, reliable, and human-relevant models
- Increase the amount of federal funding allocated to cancer prevention.

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