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Summary — Advocates of chimpanzee research claim the genetic similarity of humans and chimpanzees make them an indispensable research tool to combat human diseases. Given that cancer is a leading cause of human death worldwide, one might expect that if chimpanzees were needed for, or were productive in, cancer research, then they would have been widely used. This comprehensive literature analysis reveals that chimpanzees have scarcely been used in any form of cancer research, and that chimpanzee tumours are extremely rare and biologically different from human cancers. Often, chimpanzee citations described peripheral use of chimpanzee cells and genetic material in predominantly human genomic studies. Papers describing potential new cancer therapies noted significant concerns regarding the chimpanzee model. Other studies described interventions that have not been pursued clinically. Finally, available evidence indicates that chimpanzees are not essential in the development of therapeutic monoclonal antibodies. It would therefore be unscientific to claim that chimpanzees are vital to cancer research. On the contrary, it is reasonable to conclude that cancer research would not suffer, if the use of chimpanzees for this purpose were prohibited in the US. Genetic differences between humans and chimpanzees, make them an unsuitable model for cancer, as well as other human diseases.

Key words: *cancer, chimpanzee, neoplasm,* Pan troglodytes.

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Introduction

The United States currently holds approximately 1,000 chimpanzees in research facilities (1), and stands alone in the world as the only country actively conducting invasive research on captive chimpanzees to any significant degree. Many governments worldwide have taken legislative steps to ban or severely limit experiments on great apes predominantly for ethical reasons, based on our knowledge of the cognitive and emotional capacities of chimpanzees (2).

The ethical argument for a ban on chimpanzee research is now being progressively augmented by scientific evidence revealing *Pan troglodytes* to be a poor research model for human biology and medicine. Such evidence constituted the main rationale behind the decision of the Dutch government to disband the last chimpanzee population used for research in Europe, and to prohibit any further scientific research or testing on chimpanzees and other great apes (3). The Dutch Minister of Science, Loek Hermans, stated, "In recent years it has become clear that the need for the use of chimpanzees for research into malaria and HIV has rapidly diminished and is of limited importance. The progression of illness in chimpanzees is starkly different from that of humans, which makes the chimpanzee an unsuitable 'model'." (4).

Despite the decisions of these governments, the evidence that underpins them, as well as widespread public opinion (5), chimpanzee research remains controversial. Some advocates claim that chimpanzee experimentation is indispensable in the fight against major human diseases such as AIDS, hepatitis and cancer, without which treatments for human disease will not be realised (6). Given the ethical and financial costs of chimpanzee research, and the considerable doubt regarding its efficacy and scientific validity, these claims cannot be made lightly. Similarly, solid scientific evidence must support the opinions of those who believe chimpanzee research to be unscientific and/or unethical. To date, evidence has been published that demonstrates the redundancy and lack of scientific worth of chimpanzee research (7, 8), and that chimpanzee use in AIDS vaccine research is not predictive for human vaccine response and efficacy assessment (9). Further, studies from an ethical perspective have revealed the existence of Post-Traumatic Stress Disorder in ex-research chimpanzees now in sanctuary (10), and have detailed the physical and psychological traumas suffered by chimpanzees that have been raised in various human/chimpanzee contexts then used in research — and the chimpanzees' consequent ability to recover from such trauma once in sanctuary (11).

Cancer was chosen as the focus of this investigation because it is a major and growing contributor to the burden of human disease worldwide — as well as being a leading cause of premature death. It is responsible for a quarter of all deaths in the European Union (EU), and almost a half of deaths in the age range 45–64 years (12). In the USA, it is estimated that 1,437,180 new cancer cases and 565,650 deaths from cancer occurred in 2008 (13). Given this impact, one might expect a sound scientific argument for the use of chimpanzees in research to elucidate the molecular basis of carcinogenicity and metastasis, and in the testing of new therapeutics, evidenced by their widespread use in the past. Chimpanzees share up to 96% of our DNA (14, 15); a statistic often cited in defence of their use experimentally. As recently as 2005, it was claimed that chimpanzee research was "essential" in the testing of monoclonal antibody therapies for cancer treatment, and had been "critical" in the development of some such therapies to date (6).

In this paper, the use of chimpanzees in cancer research is described, the critical nature of such experiments and translation to human cancer treatment is deliberated, and the proposed need for chimpanzees in cancer research in the future is discussed. Diverse opinions and reasoned debate on this important topic are as crucial as the provision of new data to inform such debate.

Chimpanzee Use in Cancer Research: The Search Strategy

The database GoPubMed (16) was searched for all publications involving chimpanzees and cancer. Using the terms 'Chimpanzee[TIAB]' (locating all papers with the word 'Chimpanzee' in the title and/or abstract) and 'Neoplasms[MESH]' (restricting those chimpanzee results to all papers classified under the major disease category 'Neoplasms' in the Medical Subject Headings [MeSH] database), a comprehensive overview of cancer research involving chimpanzees and/or chimpanzee tissue/biological material was obtained. This search strategy was used because (a) it restricted results to papers that were likely to be associated with chimpanzee use more directly than a search for papers containing 'chimpanzee' *anywhere* in the text, and (b) 'neoplasms', a term very high in the hierarchical structure of MeSH headings, is more inclusive and encompasses all types of benign and malignant tumours.

Results and Analysis

This search identified 4,046 papers published between 1968 and 2008 inclusive that contained 'chimpanzee' in the title or abstract, of which 354 were classified under the MeSH term 'neoplasms' (as identified by GoPubMed, as of 20 February 2009).

A timeline was first established to determine whether there had been any increase or decrease in this research over the years (Figure 1). The first paper, published in 1968, was followed by just three further papers in the following seven years. From 1976, however, there has been a slow but steady rise in cancer-related chimpanzee publications, with an average of twelve per year between 2000 and 2007 inclusive, including an outlying increase to 22 papers in 2007. Notably, however, just three papers were listed with a 2008 publication date, and the five-year relative research interest[1] increased from 0.0014% to just 0.0017% over the past thirty years — indicating no significant growth in this type of research since the late 1970s (Figure 2; 16).

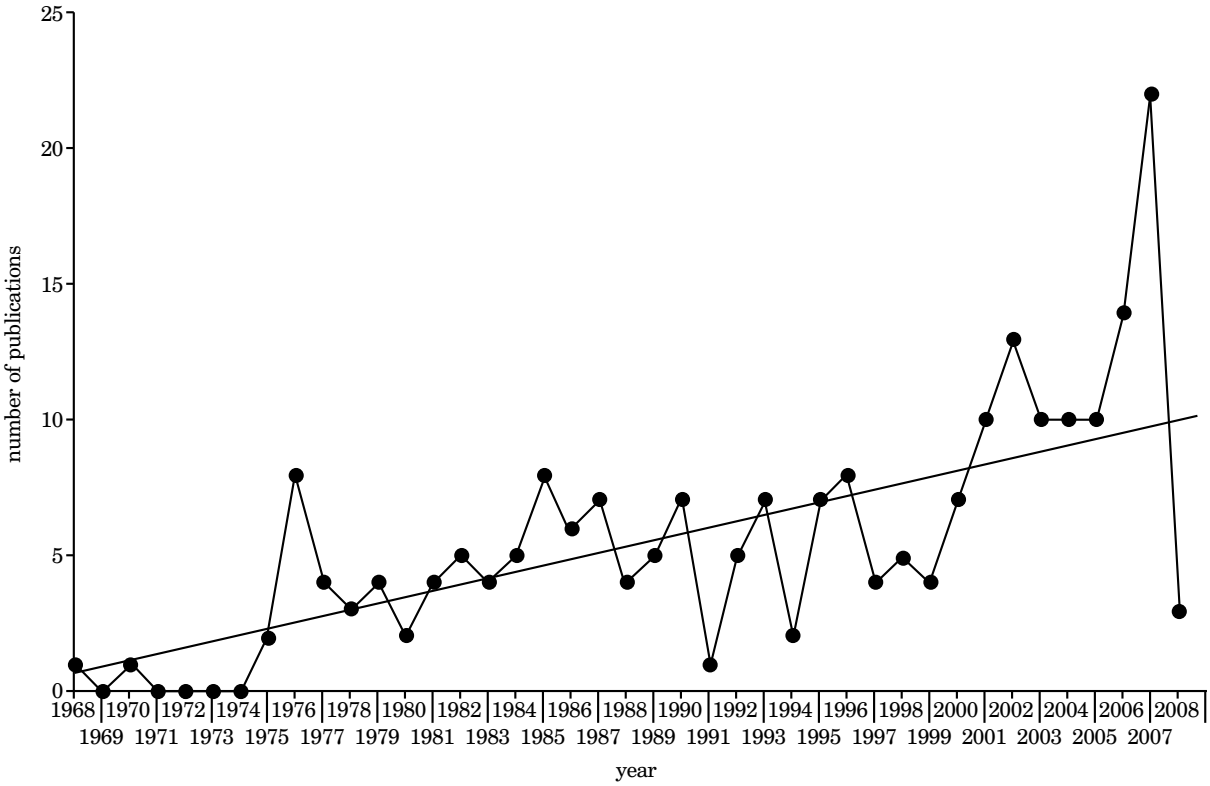
The specific areas of cancer research in which chimpanzees had been involved were then determined. The GoPubMed search described above, sorted the chimpanzee papers into categories based on the MeSH disease-categories with which they were associated. Though the categories overlap and the papers are often associated with more than one category, the data are revealing (Figure 3). There is a very 'hepatic' aspect to the results, with 51 associations with hepatitis, 43 with liver neoplasms, and 40 with hepatocellular carcinoma (HCC). In addition, there were 39 papers associated with leukaemia and 13 with T-cell leukaemia, plus 20 papers associated with melanoma. The remaining papers largely comprised of reports of tumours in chimpanzees, molecular biological investigations of genes and biochemical pathways that affect cell/tumour growth, and investigations of putative cancer therapies, among others.

Reports of chimpanzee tumours

Fourteen papers were basic reports of tumours in chimpanzees, with no direct relevance to human cancer. Seven of these papers reported malignant tumours, five described benign tumours, and two papers described tumours that can be of either type. The tumours described in these fourteen papers comprised: a report of a leiomyoma and an

[1]Weighted publications per year (subject specific)/total weighted publications per year (in PubMed).
Weighted publications per year = number of publications per year multiplied by relevance factors (as defined by PubMed).

Figure 1: A timeline to illustrate the publication of papers associated with chimpanzees and neoplasms, according to GoPubMed



The linear trendline indicates a slow but steady rise, with an average of twelve publications per year between 2000 and 2007 inclusive, including an outlying increase to 22 papers in 2007. Notably, however, just three papers were listed with a 2008 publication date.

endometrial stromal tumour (17); pulmonary myeloproliferative malignant neoplasms (18); ovarian Sertoli-Leydig cell tumour (arrhenoblastoma) (19) and fibrothecomas (20); a nasopharyngeal carcinoma (21); malignant melanoma (22); hepatocellular carcinoma (23) associated with hepatitis C virus (24) or *Schistosoma mansoni* infection (25); renal carcinoma (26); anaplastic large cell lymphoma (27); adenoma of the gallbladder (28); focal nodular hyperplasia and myelolipoma (23); gastrointestinal stromal tumour (29); and nevus lipomatosus cutaneous superficialis (30).

These reports, which were deemed worthy of inclusion in peer-reviewed journals because they were unprecedented accounts of different tumour types in chimpanzees, illustrate their rarity. This is openly acknowledged in the abstracts — for example, Porter *et al.* (23) state, "Hepatic neoplasia is rare in chimpanzees. Only four hepatic neoplasms have been reported in chimpanzees, three of which were associated with viral hepatitis."

Genetic or molecular biological investigations

Many papers described genetic and molecular biological investigations constituting 'basic research' on the genes and associated biological pathways associated with tumour development and cancer, such as cell growth/division and apoptosis (31–81). In many cases, the association with chimpanzees did not involve whole-animal research on captive animals, but instead involved the utilisation of chimpanzee tissue and/or genetic material. Typically, these studies centred on the investigation of human DNA and the characterisation of human gene splicing and/or promoter elements, and chimpanzee involvement was peripheral. For instance, chimpanzee DNA was used comparatively to obtain some form of evolutionary perspective of gene variants and architecture, or to estimate the relative importance of specific promoter elements by virtue of their temporal and inter-species conservation. Examples in more detail include:

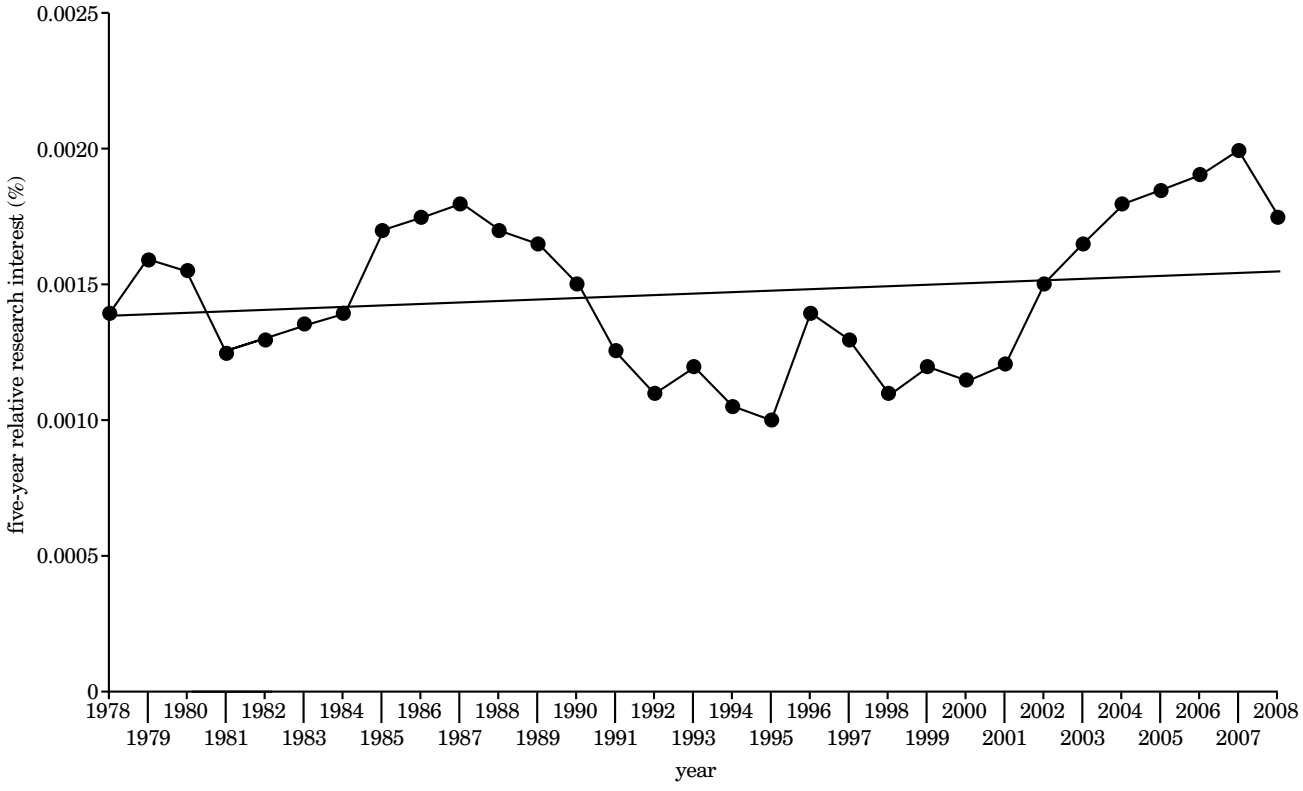
— An inactivating deletion in the human cytidine monophosphate *N*-acetylneuraminic acid hydroxylase (CMAH) gene is thought to ablate the synthesis of *N*-glycolylneuraminic acid (Neu5Gc), which is a prominent component of almost all chimpanzee cell surfaces. However, Neu5Gc is found in human cancerous cells (33), which may be the result of an alternative synthetic pathway or may be dervied from dietary sources (34).

— Analysis of the GAGE gene family, known to be expressed only in germ cells and some human tumours, revealed a cluster of 15–16 duplicate genes in humans compared to three in the chimpanzee (35).

— Examination of sequence variation in human succinate dehydrogenase (SDH) genes, which have an essential role in cellular respiration, was performed in order to gain further insights into the multiple roles of SDH in disease predisposition (36). One example includes a link between SDH and tumour susceptibility, notably between SDH mutations and familial paragangliomas (37–43). This sequence analysis utilised DNA from 48 human individuals and 18 chimpanzees (obtained from a dedicated non-human primate [NHP] DNA collection), and identified a high degree of sequence diversity in the human SDHA subunit gene compared to chimpanzee SDHA: the chimpanzee gene had 10 polymorphic variants in contrast to 21 in the human gene, and also showed 2.9-fold lower nucleotide diversity.

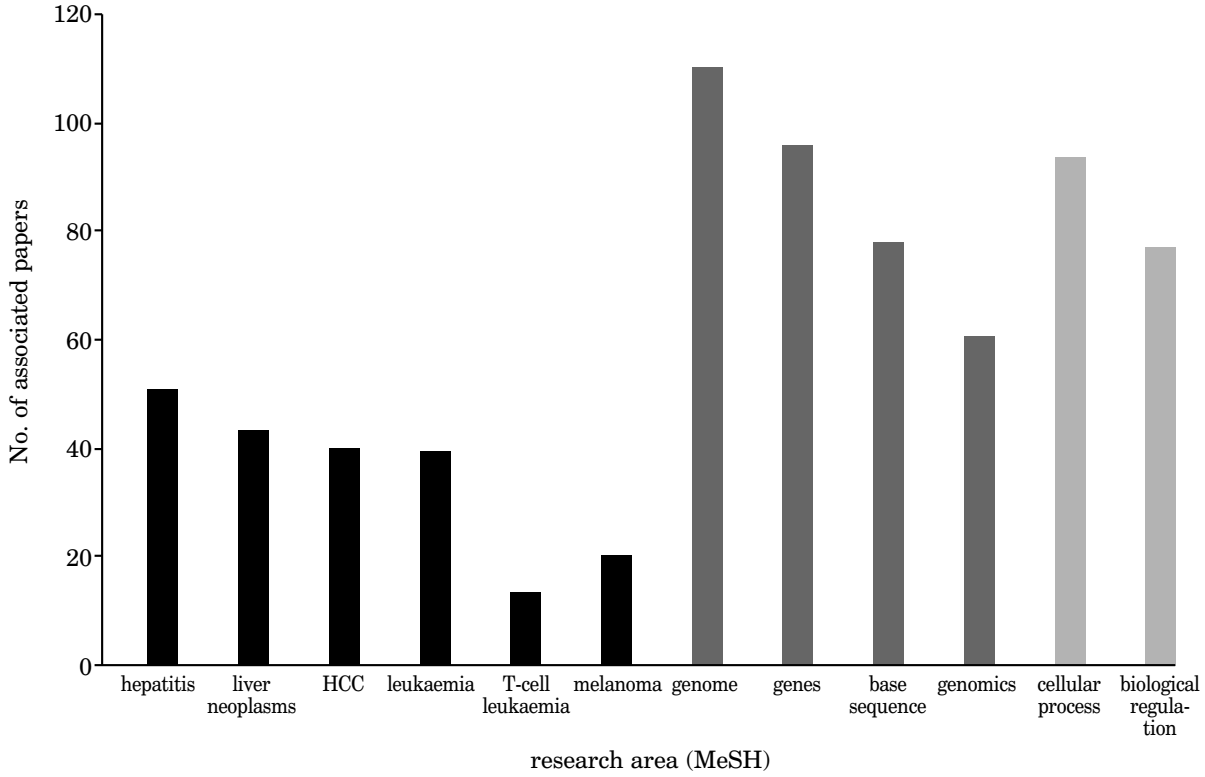
— An investigation of genetic changes in NHPs of the tumour-suppressor BRCA1 gene, which can predispose humans to breast and ovarian cancers, found that most of the BRCA1 sequence

Figure 2: A timeline and linear trendline to indicate the five-year relative research interest in papers associated with chimpanzees and neoplasms



This provides a better view of research activity and importance than an absolute number of papers, by relating publication statistics to the overall number of scientific publications indexed by PubMed, and is calculated as: Weighted publications per year (subject specific)/total weighted publications per year (in PubMed), where Weighted publications per year = number of publications per year multiplied by relevance factors (as defined by PubMed). The five-year relative research interest increased from 0.0014% to just 0.0017% during the time period over the three decades from 1978–2008 inclusive, indicating no significant growth in this type of research since the late 1970s.

Figure 3: Chimpanzee papers sorted into categories based on the MeSH disease-categories with which they were associated in GoPubMed



These categories were not exclusive, and papers were often placed into more than one category. There were 51 associations with hepatitis; 43 with liver neoplasms; and 40 with hepatocellular carcinoma (HCC). In addition, 39 papers were associated with leukaemia and 13 with T-cell leukaemia, plus 20 papers associated with melanoma. The remaining papers largely comprised of reports of tumours in chimpanzees, molecular biological investigations of genes and biochemical pathways that affect cell/tumour growth, and investigations of putative cancer therapies, among others.

= diseases; = biological sciences; = biological process.

was variable between primates (52), with pairwise identity between humans and chimpanzees of aligned segments of 93–99% (excluding indels [insertions and deletions], which are highly prevalent in non-coding regions).

— An examination of the distribution of Alu repeat sequences in the genomes of NHPs (primate-specific repetitive genomic elements, which can replicate and insert into the genome) demonstrated that identifying the phylogenetic roots of genetic disorders could explain the different susceptibility of various NHP species (including chimpanzees) to genetic diseases (76). Due to the association of Alu repeats with various types of cancer via deletions, duplications, translocations, and splice variations (76), the authors suggested similar results could be obtained for cancer, and duly published supporting data (77). This showed that the evolutionary expansion of Alu repeats to new genomic locations established new predispositions to cancer in various primate species, based on deleterious oncogenic arrangements and alternative splice sites, induced by Alu-sequence insertions. Humans have an apparent increase in Alu repeats compared to NHPs (78–80), and greater than 2,200 AluYb8-type repeats have been identified in the human genome that are absent from the chimpanzee genome (81).

— The M&M Medical Bioinformatics group in Japan published over 20 papers between 2005 and 2007, reporting comparative integromic (combined genomic, proteomic and bioinformatic) studies of signalling pathways involved

in tissue regeneration and carcinogenesis (including WNT, Notch, FGF, Hedgehog and BMP) in humans, chimpanzees, monkeys, mice and rats (82–101). Many of the pathway components examined were expressed and/or differentially regulated in diverse types of human tumours, and promoter binding-sites in component genes were highly conserved across species. In many cases in the studies by Katoh and Katoh (82–101), the similarities of the genes, proteins and promoter binding-sites under examination were high between humans and chimpanzees — as might be expected due to their close evolutionary distance.

Leukaemia research

Many of the papers in the search results associated with leukaemia did not report actual human leukaemia research. Papers were included because they were artefacts of the literature search and classification methods, and/or were due to non-direct associations with cancer; these categories were excluded from the analysis. Examples include: references to murine leukaemia viruses (102–108) and the biology of simian leukaemia viruses (109); the use of leukaemic cell lines in culture (110, 111); and papers using the old name for HIV-1, 'HTLV-III' (Human T-cell leukaemia virus, type III; 112, 113).

Several papers had a more direct association with human leukaemia research, including: an examination of the distribution of 24kDa human leukaemia-associated antigen, p24, on platelets and kidney cells, by using tissue from 12 different species including humans and chimpanzees (114); an examination of sera from 165 NHPs of different species, including chimpanzees, for the presence of antibodies to HTLV (115); investigations of the murine Friend leukaemia virus (FLV), used substantially in the past as a model for studying genetic resistance to infection by immunosuppressive retroviruses (116, 117); and a review reflecting on studies of human retroviruses in leukaemia and AIDS that cited chimpanzees in a discussion of the origin of HIV (118).

Investigation or testing of new therapies

A number of papers that cited chimpanzees in their titles and/or abstracts reported a potential new anti-cancer therapy. TNF (tumour necrosis factor)-related apoptosis-inducing ligand (TRAIL) and its receptors were profiled in human and chimpanzee tissues, to investigate recombinant TRAIL as a therapeutic anti-cancer agent (119). TRAIL and its three receptors (R1–R3) were differentially expressed in a number of organs and tissues, with notable differences between humans and chimpanzees, though the authors concluded that its lack of liver toxicity in chimpanzees was reassuring for its proposed application in humans.

Antibodies targeting the pulmonary endothelium via angiotensin-converting enzyme (ACE) were tested in tissue from ten species of primates, including humans and chimpanzees, for cross reactivity (120). Chimpanzee/human species differences were observed, and subsequent *in vivo* biodistribution studies were performed only in macaques.

Two proposed vaccines for human epithelial-cell tumours were developed and tested in chimpanzees; one based on immortalised B-cells carrying tumour-associated mucin (121), and a peptide-based MUC1 vaccine (122). The former study showed that cytotoxic T-cells recognise epitopes of mucin expressed on epithelial tumour cells, but did not include tumour challenge or rejection experiments and so no therapeutic efficacy was determined. The latter peptide approach produced positive, though transient, helper- and cytotoxic T-cell responses. An antigen-pulsed dendritic-cell (DC) approach was also explored by using chimpanzee DCs (123), in which cultured DCs are loaded *in vitro* with peptide antigens and then injected into the subject to test for immunogenic response. It was concluded, however, that MUC1-specific responses might require multiple inoculations of DCs. Chimpanzees were overlooked for subsequent experiments in favour of transgenic mice (124), which revealed that adjuvant-based peptide vaccines induced humoral but not T-cell responses with no effect on tumour growth, and that DC-based vaccines elicited tumour rejection responses in 90% of the mice tested.

The TNF-alpha pathway is strongly associated with carcinogenesis and is intensely studied for the development of therapeutics. The drug DPC333 inhibits TNF-alpha production in the blood of humans, chimpanzees and rodents (125), and its pharmacokinetics and pharmacodynamics in mice, rats, dogs and chimpanzees, as well as the results of a Phase I clinical trial in healthy humans (126), have been determined. Notable chimpanzee/human differences were reported, and further, the chimpanzee performed no better than allometric scaling with data from the other species used.

Ha6D3 monoclonal antibody was proposed for the treatment of some leukaemias, based on *in vitro* (127) and chimpanzee tests. Significant adverse events were recorded in chimpanzees, and this antibody has not been cited in any subsequent publications.

Several gene therapy investigations, in which genes are introduced directly into a patient's cells to treat a disease (often replacing a 'faulty' gene), were described. One method of gene therapy is to utilise viruses such as adenoviruses to deliver or

transduce therapeutic genes as part of their natural infectious cycle. Adenoviruses from chimpanzees have been assessed recently as an alternative to their problematic human counterparts (128–130). Chimpanzee adenoviruses have been found to transduce human dendritic cells as efficiently as human Ad5 (131–138), though there are differences in the behaviour and dispersal of DCs between humans and chimpanzees at the site of injection (139).

Twenty melanoma investigations which used chimpanzees were identified. For example, the immune response and/or tolerance of chimpanzees following exposure to different potentially therapeutic molecules was determined. A 'mutein' or altered form of human interleukin 2 (IL-2 or 'Proleukin' [aldesleukin]) — used therapeutically for advanced metastatic renal carcinoma and melanoma (140), was tested in chimpanzees, due to the severe systemic toxicity of native IL-2. (141). The mutein was better tolerated in the chimpanzee (142), but Phase I clinical (human) trial results revealed insufficient anti-tumour activity to support further evaluation (143). Purified disialoganglioside GD3 elicited a specific antibody response in chimpanzees (144, 145), but has now been replaced by other approaches, such as conjugated derivatives and anti-idiotypic GD3 mAbs, due to relatively poor immunogenicity (146–149). Finally, purified melanoma 250kDa tumour-associated antigen (TAA; also known as HMW-MAA [high molecular weight-melanoma associated antigen]) was also immunogenic in chimpanzees (149), and subsequent clinical trials produced a response in 17 of 99 patients (150). In total, over 70 clinical trials (not all involving chimpanzees) of anti-melanoma therapies took place between 1992 and 2004 (151), involving a variety of proposed therapies (152), yet advanced stage melanoma still has a "dismal prognosis" and "novel therapeutic approaches are urgently required" (151). Few therapies achieve response rates greater than 25% (153) and vaccination and augmentation of host immunity have yielded only "limited clinical success" (154).

Review papers

Several review papers were included in the results of the literature search. These publications focused on chimpanzees and cancer to varying degrees. Those specific to areas of research discussed elsewhere here have been included in their relevant sections. In addition, a review citing the low incidence of epithelial malignancy in chimpanzees, as compared to humans, proposes one basis for this difference to be changes in siglec expression/distribution and activity (155). Siglecs (sialic acid binding Ig-like lectins) recognise sialic acid molecules, one of which, Neu5Gc (*N*-glycolylneuraminic acid), is not detectable in normal human tissues but is abundant in most other mammals, including the chimpanzee (156, 157). This, and other species differences involving siglec expression, function, and sialic-acid binding preferences, and the splenic distribution of immune effector cells such as macrophages, has a significant impact on immune system function and response to infectious agents (158), and also the cell-mediated response to malignant cells (155).

A number of reviews focused on hepatitis C virus (HCV), and were included in the search results because of the association between HCV and HCC. The outcome of HCV infection is highly variable and dependent on a number of factors, however (159). HCC is not an inevitable consequence of HCV infection, nor does hepatitis research constitute cancer research *per se* — therefore these papers were excluded from detailed consideration. Notably, however, a 2003 review of HCV, including its role in HCC (160), opined that the lack of an animal model other than the chimpanzee, along with the lack of an efficient cell culture system, had hampered research in this area.

Other papers

Several papers were published on melanoma research during the 1970s, describing projects that utilised chimpanzees to generate diagnostic assays, rather than to investigate tumour biology or treatment avenues. Hyperimmunisation of chimpanzees with human melanoma cells permitted the collection of antisera that contained antibodies to common melanoma surface antigens and that were cytotoxic to melanoma cell lines (161–163). It was concluded that serologic identification could provide a means of melanoma diagnosis, though a literature search did not identify this type of approach as being in use clinically.

Following a human-based observation in which different levels of urinary excretion of lignans and isoflavinoid phytoestrogens were noted in post-menopausal breast cancer patients, their presence in the urine of chimpanzees was investigated on the basis that chimpanzees are "remarkably resistant to the carcinogenic effect of oestrogens" (164). This study confirmed that chimpanzees excrete both substances at high concentrations in their urine, and the authors suggested this could help maintain resistance against oestrogenic carcinogenicity.

Much more recently, an entirely chimpanzee-based study investigated the effect of diet on urinary excretion of these compounds (165). The results showed that diet significantly affected their excretion, with diets high in carbohydrate, protein, vegetables, and particularly fat, causing a decrease in

their elimination. Contemporary human-based studies are elucidating the effects of these compounds in people. For example, proteomic and metabonomic studies have revealed the potentially beneficial modulation of various proteins and metabolites following isoflavone consumption (166), while several epidemiological analyses have suggested no significant association between prostate or colorectal cancer risk and total serum isoflavones or lignans (167).

Development and testing of monoclonal antibody (mAb) therapies for cancer

The literature search that formed the basis of this investigation did not identify any publications reporting the use of chimpanzees in the testing of anti-cancer mAb therapies. It has been claimed, however, that chimpanzee use is essential in this respect (6). As of February 2009, almost 700 mAbs to treat various diseases including cancers were registered in the FDA's clinical trials database (*ClinicalTrials.gov*). A dedicated in-depth evaluation of these claims is therefore warranted, but is beyond the scope of this paper due to the number of mAbs involved and the confidentiality surrounding the chimpanzee data.

The salient question from the perspective of this review must be, *Is the chimpanzee ever the only "relevant" animal species and, if so, is it predictive of, and relevant to, the human response to a degree that means it is indispensable?* Given the paucity of chimpanzee data in the public domain, this is an impossible question to answer; a fact acknowledged by VandeBerg *et al.* when making their claims of chimpanzee necessity, stating, "Some of these antibodies [the 11 with FDA approval and more than 400 others in clinical trials] were tested in chimpanzees before they entered clinical trials (proprietary data, unpublished). Data for antibodies proposed for clinical trials (but not data for antibodies that produced side-effects or were ineffective in chimpanzees), are supplied to the FDA. However, these data are not published." (6).

To further illustrate this scarcity of data, a search of the *Oncology Tools* section of the website of the FDA's Center for Drug Evaluation Research (CDER: http://www.accessdata.fda.gov/scripts/cder/onctools/) was performed, using the terms 'Chimpanzee' and '*Troglodytes*.' The CDER is charged with evaluating all new drug applications in the United States before they can be sold, and analyses all drug testing data supplied to it by drug manufacturers. Its *Oncology Tools* website serves as a repository of information regarding the approval of new oncology drugs. This search produced just two results (both for infliximab [Remicade]) — a remarkably low number of results, given that several hundred mAbs have been approved or are at least in clinical trials (6), and the claims of the indispensable nature of chimpanzee testing in this field. Further, these examples were identified because the drug is associated with an increased risk of malignancies (168) not detected in preclinical studies that involved chimpanzees. The US Food and Drug Administration (FDA) pharmacological review document (169) states that chimpanzees were used in single and multiple dose safety studies because they were the only species in addition to humans whose TNF-alpha (the target of the drug) bound infliximab. Significant caveats were acknowledged however, including the lack of histopathology data and the limitation of study outcomes to clinically observable signs only. Also, while chimpanzee pharmacokinetic studies were performed, immunogenicity assessments could not be made.

It is pertinent to examine other NHP-related publications regarding cancer mAb therapies, in order to reveal which species of NHPs have been used in the development, testing and characterisation of these mAbs (presumably in preference to chimpanzees), and why. Cynomolgus monkeys, plus a small number of vervet monkeys, were used to test any (or all) of the general, developmental and reproductive toxicities, and the pharmacokinetic/pharmacodynamic properties, of rituximab (Rituxan; 170–174), tratsuzumab (Herceptin; 175, 176), alemtuzumab (Campath; 177–180), cetuximab (Erbitux; 181), bevacizumab (Avastin; 182–185) and panitumumab (Vectibix; 186). Just two chimpanzee papers were associated with these drugs, both of which were comparative genomics studies that utilised chimpanzee DNA, as cited earlier (92, 94). No NHP studies involving gemtuzumab (Mylotarg), ibritumomab tiuxetan (Zevalin) or tositumomab (Bexxar) were identified.

Notably, a search of the scientific literature by using the GoPubMed search engine (16) revealed that, of more than 65,000 papers associated with the 'Neoplasms' and 'Antibodies, monoclonal' MeSH terms, just 32 were associated with chimpanzees (0.05%). This compares to more than 55,000 associated with humans (85%), 195 papers with macaques, and 165 with vervet monkeys. Further, only two of these 32 chimpanzee papers have been published since 2002. If the use of chimpanzees in this area represented necessary and 'cutting edge' science, one might have expected the number of publications to be higher and to have increased in recent years.

Support for and citations regarding the use of chimpanzees in mAb testing were then sought in review articles on mAb therapies published in recent years. Kuroki *et al.* describe the development of native and conjugated mAb therapies and also antibody-directed gene therapies over the past decade, with no citation of chimpanzee research or testing (187). Schuster *et al.*'s general review, *Cancer Immunotherapy*, does not mention chim-

panzees (188), neither does Sharkey and Goldenberg's *Targeted Therapy of Cancer: New Prospects for Antibodies and Immunoconjugates* (189), nor Stern and Herrmann's extensive *Overview of Monoclonal Antibodies in Cancer Therapy: Present and Promise* (190).

Loisel *et al.* specifically reviewed the relevance, advantages and limitations of animal models used in cancer mAb development (191) and cited chimpanzee experiments only twice, when discussing antigen cross-reactivity and immunogenicity of therapeutic mAbs in animals. The Loisel review provided further caveats and limitations regarding the use of NHP models, including chimpanzees. The example of bevacizumab (Avastin) is given, for which serious adverse reactions, including hypertension, bleeding and thrombotic events, were not predicted by NHP models, as well as the example of trastuzumab (Herceptin), which is associated with cardiotoxicity that also was not detected preclinically (192). The review concludes that, "…it is clear that performing preclinical studies of therapeutic antibodies in animals is no more than a complex study of somewhat artificial interactions of a xenogeneic protein with the host immune system… Recent tragic events [TGN1412] show that it is very difficult to circumvent many of those drawbacks, and all these animal models [including chimpanzees] must be considered as models only."

The example of TGN1412, a monoclonal superagonist of the CD28 T-cell receptor intended for the treatment of B-cell chronic lymphocytic leukaemia (193), illustrates the difficulties of extrapolating preclinical data to humans — even from NHPs that demonstrate extremely encouraging pharmacokinetic and toxicological data and close homology of all relevant molecules. Though tested in rhesus and cynomolgus monkeys and not chimpanzees, TGN1412 induced a systemic inflammatory response in all six volunteers taking it in first-in-human trials, despite being administered at a subclinical dose some 500 times lower than the dose found to be safe in animals (194). Multiple cytokine release syndrome ensued, leading to multiple organ failure and, in some cases, cardiovascular shock and acute respiratory distress syndrome (195) — in complete contrast to the NHP preclinical safety data.

In May 2006, a workshop was conducted by the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) entitled, *Opportunities for Reducing the Use of Non-human Primates in the Development of Monoclonal Antibodies* (196). The report from this workshop echoed concerns voiced over TGN1412 elsewhere, and identified opportunities to avoid NHP use altogether in mAb development. They cautioned that, while using chimpanzees *might* be scientifically relevant, the "considerable ethical concerns regarding the use of chimpanzees" mean that "many in the pharmaceutical industry do not see this as an option." Data exist for 16 mAbs that were licensed in the EU at the time of publication, but just one of these, infliximab (Remicade), was tested in chimpanzees, as discussed earlier. Notably, the mouse was deemed the most relevant species, alongside the chimpanzee, which was used with surrogate antibodies to provide preclinical safety and efficacy data.

The author of the NC3Rs report subsequently published a review on the subject of species relevance in mAb testing, which concluded that, "…the assumption that a shift from Old World primates towards the use of chimpanzees might overcome some of the issues associated with species relevance is not necessarily supported by experts or evidence. For example, some of the effects of TGN1412 might have also been masked in the chimpanzee owing to the human-specific loss of expression of CD33-related Siglecs…also, the use of chimpanzees for preclinical studies is restricted by scientific, logistical and ethical problems, suggesting that the chimpanzee might be of limited value in the development of mAbs" (197).

Discussion and Conclusions

One would expect chimpanzees to have been used widely in many, if not all, areas of cancer research, due to ubiquitous claims of genetic similarity to humans — a similarity that, *prima facie*, would seem to underpin their suitability and relevance as a model species for human disease research. This genetic similarity forms the basis of recent claims, for example, of the indispensability of chimpanzees for the testing of monoclonal antibody therapies (including those for cancer treatment), and for their critical involvement in the development of some such therapies to date (6).

A wider view of the nature of chimpanzee use, however, would indicate that chimpanzees simply cannot constitute a vital part of research into cancer, or indeed any other disease. Firstly, the general importance of chimpanzee research was assessed via an extensive citation analysis of papers reporting chimpanzee data, and a detailed evaluation of the contribution such papers have made to human clinical progress and practice. Half of a statistically-significant sample of 95 chimpanzee papers had not been subsequently cited at all. A further 35% were cited only by papers that did not describe well-developed prophylactic, diagnostic or therapeutic methods for combating human diseases. Less than 15% of chimpanzee studies had been cited by papers that were relevant to human medicine. An in-depth analysis of these studies revealed that the chimpanzee experiments had contributed very little, if anything at all, to the outcome of those papers reporting an advance in human clinical practice (7, 8). Secondly,

of the approximate 1,000 chimpanzees remaining in research laboratories in the United States, it is believed only around 20% are in 'active research protocols' and are merely being 'warehoused' (*personal communication*, J.L. VandeBerg (2008), at the International Primatological Society Meeting, Edinburgh, UK). Thirdly, over the last decade, approximately 600 have been retired to sanctuaries (198) and, in 2007, the US National Center for Research Resources (NCRR), an institute under NIH jurisdiction, chose to end federal funding for breeding NCRR-owned or supported chimpanzees for research, thus making the previous 10-year voluntary breeding moratorium permanent (199). Finally, throughout the world, there has been a steady and growing number of modern, scientifically advanced countries that have limited, terminated or banned the use of chimpanzees and other great apes in research (2). Most recently, in 2009, an EU-wide ban on the use of chimpanzees and other great apes was passed (200). None of these trends would be seen if their use in research, cancer or otherwise, were crucial.

Further, and more specifically, this review determined that chimpanzees have scarcely been used in any form of cancer research and that chimpanzee tumours are both rare and biologically different from human cancers. Papers describing potential new cancer therapies tested in chimpanzees often included significant caveats based on species differences, acknowledged that the chimpanzee model performed no better than other animal models, and/or described interventions that had not been pursued clinically, presumably due to adverse preclinical results. Notably, such studies used very few chimpanzees — typically just four animals per study (an average obtained from papers cited in this review). This small sample size casts doubt on the scientific significance of data obtained from this research in any case. In the field of mAb development, where chimpanzees have been lauded as being of crucial importance, available evidence clearly indicates that this is not the case. Profound species differences in carcinogenicity, cell growth, apoptosis and metastasis demonstrate that chimpanzees constitute a poor research model for human cancer, despite their overall genetic similarity to humans.

Many papers, such as the series published by Katoh & Katoh (82–101), described comparative genomic studies in which human genes involved in molecular pathways associated with cell growth/differentiation and apoptosis were compared to genes from other species, including chimpanzees. Chimpanzee DNA was not central to these studies, in which gene expression was analysed between cancerous and normal tissue, and promoter-binding sites, sequences and splicing were compared between humans and a variety of other species for evolutionary conservation, for example. While it may be argued that this type of investigation can be of relevance to human tumour biology, it has no bearing on the use of captive chimpanzee populations in laboratory research, as they utilise readily available genetic material only.

Few papers described the investigation of new therapeutic interventions, but these are of interest due to poor interspecies extrapolation. Examples reported include: recombinant TNF-related apoptosis-inducing ligand (119); inhibitor of TNF-alpha converting enzyme (126); anti-angiotensin-converting enzyme antibodies (120); cell-based vaccines (121, 123, 124, 139); and therapies targeting various solid tumours, melanoma, and leukaemias (see *Results and Analysis* section). No publications were identified that described chimpanzee use in the development or testing of mAb cancer therapies.

The underlying reasons for the lack of relevance of chimpanzees to human cancer research, and thus for their lack of utility and adoption as a cancer research model, lie in numerous fundamental yet far-reaching genetic differences between chimpanzees and humans that betray superficial claims of genetic similarity. The consequences of differences in the prevalence of Alu sequences, siglec expression, and other genetic differences are described in detail in the *Results and Analysis* section. Yet there are further differences: at least twenty genes implicated in human cancers, some of which are definitively involved in tumour formation, are significantly different in chimpanzees (48); other significant differences have been identified in protease genes, many of which affect the immune system and that therefore have a potential bearing on tumour establishment and growth (201); 80% of orthologous proteins differ between humans and chimpanzees, including proteins linked to breast cancer (202); and 6%–8% of orthologous exons display pronounced differences in splicing, which affects diverse functions including gene expression, signal transduction, cell death, immune defence, and susceptibility to certain diseases, including cancers (203).

A recent structural genomics study, which compared the regulation of apoptosis between humans and chimpanzees (204), acknowledged that nutritional and ecological differences contributed to changes in cancer incidence between the species, but could not "coherently explain" an order of magnitude increase in cancers of the breast, ovary, lung, stomach, colon and rectum in humans (205). Instead, the authors implicated some of the estimated 40 million differences (of various types) between the human and chimpanzee genomes, which determine susceptibility and tolerance — as seen in different human populations (206). The examination of around 500 proteins involved in apoptotic or DNA-repair pathways was revealing. Many protein-coding regions were organised differently across the chromosomes of each of the species. Some 5% of proteins analysed

were expressed from genes with different numbers of exons/different splice variants between the two species, while more than 80 proteins from genes with identical intron numbers were the products of genes that had longer introns in chimpanzees — and therefore probably more regulatory regions and more regulatory RNA molecules affecting gene splicing and expression. Around one tenth of the genes involved in the analysis might be pseudogenes in the chimpanzee. Further, there were, on average, more than 2.5 splice variants per gene in the chimpanzee, compared to 1.5 in humans, human proteins contained a greater number of post-translational modification sites than the corresponding chimpanzee proteins, and, despite a mean protein identity of 96% between species, many proteins contained changes that altered important protein–protein interactions and/or compound-binding sites. To paraphrase the authors' conclusions, "a complex pattern of subtle variances and a few large-scale changes on different levels of chromosome organisation, gene structure, post-transcriptional and post-translational modifications to functional changes in protein structures" is responsible for the wholesale changes in carcinogenicity between humans and chimpanzees.

It would therefore seem that Russell & Burch's "high-fidelity" fallacy (207) — the mistaken notion that the more a model superficially resembles the thing being modelled, the more suitable it is for elucidating the phenomenon in question — is highly applicable to cancer research in chimpanzees, and indeed to chimpanzee research on human diseases more generally. When our closest genetic relative has contributed so little to combating cancers that have cost hundreds of millions of lives and hundreds of billions of research dollars, it is unscientific to claim that they must remain a crucial and necessary tool in cancer research — even in contemporary testing of mAb therapies. To the contrary, there is no valid evidence to support their use in the future, and it is reasonable to conclude that cancer research would not suffer if the use of chimpanzees for this purpose were prohibited in the USA.

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