



InFocus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology



Nature Publishing Group is a division of the international scientific publishing company Springer Nature that publishes academic journals, magazines, online databases, and services in science and medicine. Nature Publishing Group's flagship publication is *Nature*, a weekly multidisciplinary journal first published in 1869.

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The world's largest multidisciplinary scientific society and a leading publisher of cutting-edge research through its Science family of journals, AAAS has individual members in more than 91 countries around the globe. The AAAS seeks to "advance science, engineering, and innovation throughout the world for the benefit of all people."

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Inside:

✓ A New Face in the CCMP

✓ The end of an era for MoHo

The NIH Principles & Guidelines for Reporting Preclinical Research: An Overview

The advancement of science relies on research that is rigorous (e.g. robust and unbiased) in its experimental design, analysis, and interpretation, and reproducible in terms of research findings⁵. Furthermore, it is fundamental that studies are reported in sufficient detail to allow the scientific community, research funding agencies and disease advocacy organizations to evaluate the reliability of research results⁴. In recent years there has been a growing awareness of the need for rigorously designed published preclinical studies, to ensure that such studies can be reproduced. In light of this, the NIH held a joint workshop with the Nature Publishing Group and the American Association for the Advancement of Science with journal editors representing over 30 basic/preclinical science journals in which NIH-funded investigators have most often published⁵. The main focus of the workshop was to identify areas in biomedical research where rigor,

reproducibility, robustness, and transparency could be improved. The product of this effort was the creation of a set of principles that would facilitate these goals, which a considerable number of journals such as PLOS ONE, Cell, Nature, and Science have agreed to endorse. Furthermore, the NIH encourages research centers and journals to expand on these principles and adapt them to fit the unique needs and challenges of each research field. For example, the Biophysical Journal and the Center for Open Science have created guidelines using these principles as a model^{1,2}. The set of principles is listed below

1. Rigorous statistical analysis: The results of most experiments should be assessed by an appropriate statistical analysis and the analysis should reflect the purpose of the study³. The general aim is to extract all of the useful information present in the data in a way that it can be interpreted, taking account

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Endogenous Murine Retroviruses: What you need to know!

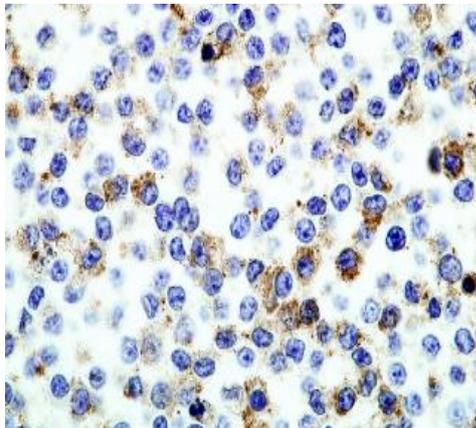
Approximately 40% of the mouse's genome is occupied by retroelements such as retroviruses, and not surprisingly, these elements vary among mouse strains and stocks^{4,10}. As these endogenous (originating from within the host) elements are integrated into the genome, they are transmitted to offspring. In general they are nonpathogenic; however, they may elicit an immune response in the host throughout life or can result in neoplasia. Of the endogenous retroviruses, the gamma retrovirus, murine leukemia virus (MuLV), has been the most studied. MuLV has been shown to be closely related to its exogenous (originating from outside the host) counterparts and is capable of being expressed without using host transcriptional elements^{1,7}.

Exogenous MuLVs, found in wild mouse species but not in laboratory mice, have been implicated in inducing lymphomas, hindlimb paralysis, and altering coat color or texture^{1,7}. Laboratory mice are largely

resistant to the virus, which is mainly transmitted from dam to offspring through milk. Two exogenous MuLVs, the Abelson and Moloney viruses, are capable of usurping host DNA, and occasionally inducing altered cell division with rapid transformation resulting in neoplasia and thus are used as experimental tools¹. Retroviruses such as MuLV, are capable of inducing tumors by two mechanisms, either by containing and transmitting an oncogene or integrating into and mutating a proto-oncogene in the host genome. For example, the AKR mouse strain develops thymic lymphomas of T-cell origin by 6-12 months of age and BALB/c mice develop late-onset multicentric lymphoma via the latter mechanism^{1,8}.

The tropism of endogenous MuLVs determines which species or cell lines the virus is capable of infecting. Ecotropic MuLVs infect only mice or murine derived cell lines. In contrast, polytropic MuLVs are capable of

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The expression of MuLV p30 in RAW264.7 cells. By Janet Hartley.

Credit: [https://microbewiki.kenyon.edu/index.php/Murine_Leukemia_Virus_\(MuLV\)](https://microbewiki.kenyon.edu/index.php/Murine_Leukemia_Virus_(MuLV))

The NIH Principles & Guidelines for Reporting Preclinical Research *Cont. from pg. 1*



Please WELCOME
Dr. Kvin Lertpiriyapong
CCMP's new Senior Clinical
Veterinarian

Dr. Kvin Lertpiriyapong recently joined the CCMP as a Senior Clinical Veterinarian in RARC's Veterinary Services section. Kvin obtained his BA in integrative Biology and Ph.D. in Plant Biology from UC Berkeley. His doctoral dissertation investigated the genetic mechanisms regulating plant cell growth. These experiences gave him a strong foundation in the biological research and molecular biology and genetics. He then pursued a doctorate in veterinary medicine at Western University of Health and Sciences, where he also developed a strong interest in Comparative/Laboratory Animal Medicine. After graduation in 2010, he pursued postdoctoral training in Comparative Medicine at the Massachusetts Institute of Technology where he conducted research in gastrointestinal cancer and GI bacterial pathogenesis using animal models. He has been a Diplomate of the American College of Laboratory Animal Medicine since 2015.

Dr. Lertpiriyapong will oversee the provision of clinical care for rodents housed at the Rockefeller Research Laboratory, in addition to providing clinical support for human xenograft and aquatic models at both MSK and WCM. With his knowledge in molecular biology and genetics and his strong record in collaborative research, Kvin is looking forward to providing research support to investigators utilizing animal models and is excited to join MSK and WCM.

of biological variability and measurement error. The materials and methods section should describe the statistical methods used in analyzing the results providing enough detail to allow verification of results using the original data. As an example, the guidelines used by the Biophysical Journal specify that any statistical analysis must be checked for accuracy and if statistical software is used, the source (including version number) of the tools must be listed in the Materials and Methods¹. Statistics should be fully reported in the manuscript/article, including the statistical test used, exact value of N and the definitions of center and dispersion and the precision measures (e.g., mean, median, SD, SEM and confidence intervals). For journals, a section outlining the journal's policies for statistical analysis should be included in the Information for Authors, and the journal should have a mechanism to check the statistical accuracy of submission⁵.

2. Transparency in reporting: To ensure that published studies can be replicated, authors should adhere to the following core set of principles³:

a. **Standards:** Use of community-based standards (such as nomenclature and reporting standards like the ARRIVE Guidelines (presented in the prior In Focus issue).

b. **Replicates:** Report how often each experiment was performed and whether the results were validated under a range of conditions. Sufficient information about sample collection must be provided to distinguish between independent biological data points and technical replicates.

c. **Statistics:** Require that statistics be fully reported in the paper.

d. **Randomization:** State whether the samples were randomized and specify the method(s) used. Treatments should be assigned so that each experimental unit has a known, often equal, probability of receiving a given treatment. Randomization is essential because there are often sources of variation, known or unknown, which could bias the results.

e. **Blinding:** State whether experimenters were blind to group assignment and outcome assessment. To avoid bias, experiments should be performed "blind"

with respect to treatment(s). After the randomized allocation of experimental units to the treatment groups, animals/samples/treatments should be coded until the data are analyzed.

f. **Sample-size estimation:** State whether an appropriate sample size was computed when the study was being designed and include the statistical method of computation. If power analysis was not used, include how the sample size was determined.

g. **Data handling:** State the criteria that were used for exclusion of any data/results or subjects, especially if the results do not support the main findings of the study. Describe any outcomes or conditions that were measured or used and are not reported in the results section. Researchers should also consider, in advance, the rules for ceasing data collection and how outliers will be defined and handled.

3. **Data and material sharing:** All datasets on which the conclusions of the paper rely should be made available upon request (where ethically appropriate) during consideration of the manuscript (by editors and reviewers). Deposition of datasets in public repositories and material sharing after publication should be required.

4. **Consideration of refutations:** Journals should have a policy stating that if the journal publishes a paper, it assumes responsibility to consider publication of refutations of that paper, according to its usual standards of quality.

5. **Consider establishing best practice guidelines for:** Image based data as well as for biological material, in particular for:

a. **Antibodies:** report source, characteristics, dilutions and how they were validated

b. **Cell lines:** report source, authentication and mycoplasma contamination status

c. **Animals:** report source, species, strain, sex, age, husbandry, inbred and strain characteristics of transgenic animals

The collaboration of all stakeholders such as investigators, reviewers, funding agencies, and journals will be essential for the improvement of transparency and

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Farewell to MoHo (Long Island City Vivarium)

After almost 13 years of continuous operations, MSK has decided to close its LIC Vivarium (LIC). Since its opening in early 2004, LIC supported the creation, breeding and maintenance of hundreds of thousands of genetically engineered mice, which have been critical tools in many important scientific discoveries. Moreover, it served as the home of the bi-institutional Mouse Genetics Core (MGC). During the completion of the first and second phases of the Zuckerman Research Center (ZRC) Vivarium with concomitant closure of the Kettering Laboratory, the facility allowed accommodation of many mouse colonies during a period of marked program growth. Importantly, the design and operational concepts employed at LIC served as the model for both the ZRC's and Belfer Research Building's (BRB) state-of-the-art vivaria.

All mouse colonies are now housed in Manhattan. The MGC's Colony Management Group (CMG) and the mouse colonies they maintain are located in the C4 level of the ZRC and the Transgenics Group have been relocated to the 12th floor of the Rockefeller Research Lab building. The MGC's genotyping service has been discontinued; this service is now performed by Transnetyx, a company providing automated genotyping services to academia, government, and industry. Additional information on using Transnetyx's services will be forthcoming. The MGC's Molecular Biology Group, relocated to ZRC-966, will continue to assist users in the preparation of DNA and CRISPR for generating transgenic and knockout animals.

The CCMP thanks all of our staff and colleagues that helped make LIC a remarkable success.

Endogenous Murine Retroviruses: What you need to know! *Cont. from pg. 1*

infecting the natural mouse host as well as a range of other species, including humans. Xenotropic MuLV (XMLV) is incapable of replicating in mice but can infect cells of other species including humans. This latter subset of MuLVs pose the greatest risk to humans, namely as zoonoses (diseases transmitted from animals to humans).

It is important to consider the impact of endogenous XMLV infection when passaging human xenografts in mice. When implanted, patient-derived xenografts (PDX) are inevitably supported by murine stromal elements such as vascular and fibrous networks, which contain murine DNA and thus endogenous viral elements. Both murine DNA and its cognate viral elements are capable of persisting in culture for long periods of time. A recent study addressed how frequently XMLV contaminates human xenografts and can spread to human cell lines including small cell lung cancer, several hematologic tumors, prostatic tumors, urogenital tumors, and pancreatic tumors maintained in vitro¹¹. These authors demonstrated that XMLV is common in cultures initiated from PDX passaged in mice and this cannot be solely attributed to contamination with murine DNA (26% of cell cultures tested were positive for MuLV DNA sequences without murine DNA contamination). Furthermore, 17% of naïve, non-xenograft cell cultures merely maintained in the same facility as the infected xenograft cultures were also positive for MuLV sequences, indicating that passage through mice was not necessary for the virus to infect cell lines.

Whether XMLV has the potential of causing disease in humans following contact with tissue of fluids from XMLV-infected animals or xenografts remains unknown. However, there have been reports of outbreaks of other viruses associated with human xenografts. For example, staff at a medical center became infected with lymphocytic choriomeningitis virus (LCMV)², a zoonotic virus that causes fever and myalgia, after handling Syrian hamsters implanted with LCMV-infected cells.

A link between XMLV and prostate cancer, as well as chronic fatigue syndrome, has been proposed. However, the methods used to detect the virus may

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The outside of 39-40 Crescent Street may not have impressed but what happened inside was awe inspiring!



State of the art laboratory space supported the Mouse Genetics Core



Custom high volume ventilated mouse caging systems.



THANKS to the dedicated staff that supported the LIC “MOuse HOuse” and our institutions’ research efforts for ~ 13 years!

Endogenous Murine Retroviruses, Cont. from pg. 3

have been flawed^{4,5,6} and the conclusions drawn from these studies should be scrutinized as the reagents used may have been contaminated with murine DNA, which may contain endogenous MLV and create false positive results⁹.

The need for screening assays to detect endogenous MuLV in both human and murine tissues used as cell lines for xenotransplantation is justified, albeit complicated³. The supporting murine stromal components mentioned previously may spontaneously release infectious endogenous virus, posing a threat to the xenografts, but also to staff handling them. Additionally, the use of immunocompromised animals for xenotransplantation invariably alters host-viral interactions, with engraftment potentially activating endogenous virus. These animals may contribute to the undetected spread of the virus. Of considerable concern was the observation that human cell lines never passaged through mice, yet handled in the same facility as XMLV-infected human cell lines, became infected within the laboratory in a period as short as several days¹¹. Because it is unclear how the virus spreads from cell line to cell line, or if animals are a potential infectious source, working with human xenografts in a Class II biosafety cabinet (BSC) is crucial. At MSK and WCM, xenografted animals and human-derived cell lines must always be handled in a Class II BSC, donning the appropriate personal protective equipment (PPE). The significance of XMLV as a biological hazard to laboratory personnel remains unclear yet reducing the potential for exposure when working with xenograft animals and cell lines is prudent¹¹.

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-Mariya Gugel, DVM

The NIH Principles & Guidelines for Reporting,

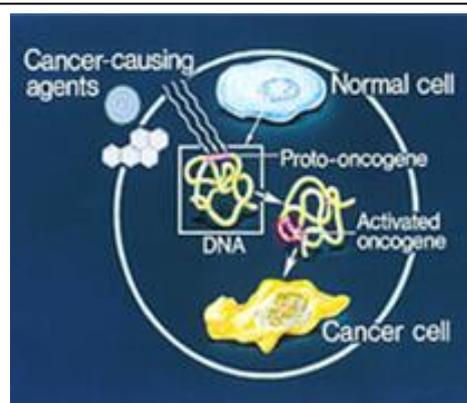
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quality of reporting⁴. Investigators are encouraged to provide relevant information about the design, execution and analysis of experiments and reviewers should consider these issues in their assessments. This effort should then increase the overall quality and applicability of preclinical research.

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1. Biophysical Society. 2015. Guidelines for the reproducibility of biophysics research. Biophysical Journal (<http://www.cell.com/pb/assets/raw/journals/society/biophysj/PDFs/reproducibility-guidelines.pdf>).
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- Christopher Cheleuitte, DVM, PhD



Most normal cells undergo apoptosis when critical functions are altered. Activated oncogenes can cause those cells to survive and proliferate instead. Most oncogenes require an additional step, such as mutations in another gene, or environmental factors, such as viral infection, to cause cancer.

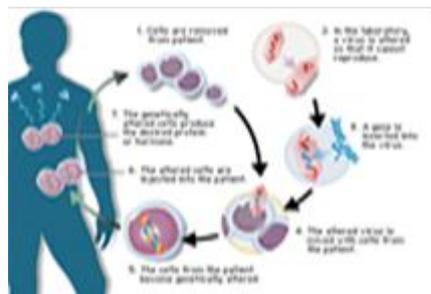
Image/caption credit: <https://en.wikipedia.org/wiki/Oncogene>

Some add-ons to the genetics lexicon:

* **RETROELEMENTS:** A sequence of DNA integrated into a specific location within the genome via an RNA intermediate that was reverse-transcribed.

* **ONCOGENE:** A gene that can transform a cell into a tumor cell under certain conditions

* **PROTO-ONCOGENE:** A gene which becomes an oncogene when altered; typically responsible for signals involving cell division or cell death



It has been determined the best systematic treatment via gene therapy would be using a retrovirus, such as HIV or MuLV, capable of gene delivery to specific cells in vivo and efficiently expressing the desired gene in the cells.

Image/caption credit: [https://microbewiki.kenyon.edu/index.php/Murine_Leukemia_Virus_\(MuLV\)](https://microbewiki.kenyon.edu/index.php/Murine_Leukemia_Virus_(MuLV))