



# Virology under the Microscope—a Call for Rational Discourse

 Felicia Goodrum,<sup>a</sup> Editor in Chief, *Journal of Virology*,  Anice C. Lowen,<sup>b</sup>  Seema Lakdawala,<sup>b</sup>  James Alwine,<sup>a,c</sup>  Arturo Casadevall,<sup>d</sup> Editor in Chief, *mBio*,  Michael J. Imperiale,<sup>e</sup> Editor in Chief, *mSphere*,  Walter Atwood,<sup>f</sup>  Daphne Avgousti,<sup>g</sup>  Joel Baines,<sup>h</sup>  Bruce Banfield,<sup>i</sup>  Lawrence Banks,<sup>j</sup>  Sumita Bhaduri-McIntosh,<sup>k</sup>  Deepta Bhattacharya,<sup>l</sup>  Daniel Blanco-Melo,<sup>g</sup>  David Bloom,<sup>k</sup>  Adrianus Boon,<sup>p</sup>  Steeve Boulant,<sup>k</sup>  Curtis Brandt,<sup>n</sup>  Andrew Broadbent,<sup>o</sup>  Christopher Brooke,<sup>p</sup>  Craig Cameron,<sup>q</sup>  Samuel Campos,<sup>l</sup>  Patrizia Caposio,<sup>r</sup>  Gary Chan,<sup>s</sup>  Anna Cliffe,<sup>t</sup>  John Coffin,<sup>u</sup>  Kathleen Collins,<sup>v</sup>  Blossom Damania,<sup>q</sup>  Matthew Daugherty,<sup>w</sup>  Kari Debbink,<sup>x</sup>  James DeCaprio,<sup>y</sup>  Terence Dermody,<sup>z</sup>  Jimmy Dikeakos,<sup>aa</sup>  Daniel DiMaio,<sup>bb</sup>  Rhoel Dinglasan,<sup>k</sup>  W. Paul Duprex,<sup>z</sup>  Rebecca Dutch,<sup>cc</sup>  Nels Elde,<sup>dd</sup>  Michael Emerman,<sup>g</sup>  Lynn Enquist,<sup>ee</sup>  Bentley Fane,<sup>l</sup>  Ana Fernandez-Sesma,<sup>ff</sup>  Michelle Flenniken,<sup>gg</sup>  Lori Frappier,<sup>hh</sup>  Matthew Frieman,<sup>o</sup>  Klaus Frueh,<sup>r</sup>  Michaela Gack,<sup>ii</sup>  Marta Gaglia,<sup>n</sup>  Tom Gallagher,<sup>jj</sup>  Denise Galloway,<sup>g</sup>  Adolfo García-Sastre,<sup>ff</sup>  Adam Geballe,<sup>g</sup>  Britt Glaunsinger,<sup>kk</sup>  Stephen Goff,<sup>ll</sup>  Alexander Greninger,<sup>mm</sup>  Meaghan Hancock,<sup>r</sup>  Eva Harris,<sup>kk</sup>  Nicholas Heaton,<sup>nn</sup>  Mark Heise,<sup>q</sup>  Ekaterina Heldwein,<sup>u</sup>  Brenda Hogue,<sup>oo</sup>  Stacy Horner,<sup>nn</sup>  Edward Hutchinson,<sup>pp</sup>  Joseph Hyser,<sup>qq</sup>  William Jackson,<sup>oo</sup>  Robert Kalejta,<sup>nn</sup>  Jeremy Kamil,<sup>rr</sup>  Stephanie Karst,<sup>k</sup>  Frank Kirchhoff,<sup>ss</sup>  David Knipe,<sup>y</sup>  Timothy Kowalik,<sup>tt</sup>  Michael Lagunoff,<sup>mm</sup>  Laimonis Laimins,<sup>uu</sup>  Ryan Langlois,<sup>vv</sup>  Adam Luring,<sup>v</sup>  Benhur Lee,<sup>ff</sup>  David Leib,<sup>ww</sup>  Shan-Lu Liu,<sup>xx</sup>  Richard Longnecker,<sup>uu</sup>  Carolina Lopez,<sup>m</sup>  Micah Luftig,<sup>nn</sup>  Jennifer Lund,<sup>g</sup>  Balaji Manicassamy,<sup>yy</sup>  Grant McFadden,<sup>oo</sup>  Michael McIntosh,<sup>k</sup>  Andrew Mehle,<sup>n</sup>  W. Allen Miller,<sup>zz</sup>  Ian Mohr,<sup>aaa</sup>  Cary Moody,<sup>q</sup>  Nathaniel Moorman,<sup>q</sup>  Anne Moscona,<sup>ll</sup>  Bryan Mounce,<sup>jj</sup>  Joshua Munger,<sup>bbb</sup>  Karl Münger,<sup>u</sup>  Eain Murphy,<sup>s</sup>  Mojgan Naghavi,<sup>uu</sup>  Jay Nelson,<sup>r</sup>  Christopher Neufeldt,<sup>ccc</sup>  Janko Nikolich,<sup>l</sup>  Christine O'Connor,<sup>ddd</sup>  Akira Ono,<sup>v</sup>  Walter Orenstein,<sup>ccc</sup>  David Ornelles,<sup>eee</sup>  Jing-hsiung Ou,<sup>fff</sup>  John Parker,<sup>h</sup>  Colin Parrish,<sup>h</sup>  Andrew Pekosz,<sup>x</sup>  Philip Pellett,<sup>ggg</sup>  Julie Pfeiffer,<sup>hhh</sup>  Richard Plemper,<sup>iii</sup>  Stephen Polyak,<sup>mmm</sup>  John Purdy,<sup>l</sup>  Dohun Pyeon,<sup>jjj</sup>  Miguel Quinones-Mateu,<sup>aa</sup>  Rolf Renne,<sup>k</sup>  Charles Rice,<sup>kkk</sup>  John Schoggins,<sup>hhh</sup>  Richard Roller,<sup>yy</sup>  Charles Russell,<sup>lll</sup>  Rozanne Sandri-Goldin,<sup>mmm</sup>  Martin Sapp,<sup>rr</sup>  Luis Schang,<sup>h</sup>  Scott Schmid,<sup>nnn</sup>  Stacey Schultz-Cherry,<sup>lll</sup> Editor in Chief, *Journal of Virology*,  Bert Semler,<sup>mmm</sup>  Thomas Shenk,<sup>ee</sup>  Guido Silvestri,<sup>cc</sup>  Viviana Simon,<sup>ff</sup>  Gregory Smith,<sup>uu</sup>  Jason Smith,<sup>mmm</sup>  Katherine Spindler,<sup>vv</sup>  Megan Stanifer,<sup>k</sup>  Kanta Subbarao,<sup>ooo</sup>  Wesley Sundquist,<sup>ddd</sup>  Mehul Suthar,<sup>ccc</sup>  Troy Sutton,<sup>ppp</sup>  Andrew Tai,<sup>v</sup>  Vera Tarakanova,<sup>qqq</sup>  Benjamin tenOever,<sup>aaa</sup>  Scott Tibbetts,<sup>k</sup>  Stephen Tompkins,<sup>rrr</sup>  Zsolt Toth,<sup>k</sup>  Koenraad van Doorslaer,<sup>l</sup>  Marco Vignuzzi,<sup>sss</sup>  Nicholas Wallace,<sup>ttt</sup>  Derek Walsh,<sup>uu</sup>  Michael Weekes,<sup>uuu</sup>  Jason Weinberg,<sup>v</sup>  Matthew Weitzman,<sup>vvv</sup>  Sandra Weller,<sup>www</sup>  Sean Whelan,<sup>m</sup>  Elizabeth White,<sup>vvv</sup>  Bryan Williams,<sup>xxx</sup>  Christiane Wobus,<sup>y</sup>  Scott Wong,<sup>r</sup>  Andrew Yurochko<sup>rr</sup>

<sup>a</sup>Department of Immunobiology, BIOS Institute, University of Arizona, Tucson, Arizona, USA

<sup>b</sup>Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia, USA

<sup>c</sup>Department of Cancer Biology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>d</sup>Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

<sup>e</sup>Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, USA

<sup>f</sup>Brown University, Providence, Rhode Island, USA

<sup>g</sup>Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

<sup>h</sup>Cornell University, Ithaca, New York, USA

<sup>i</sup>Queen's University, Kingston, Ontario, Canada

<sup>j</sup>International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

<sup>k</sup>University of Florida, Gainesville, Florida, USA

<sup>l</sup>University of Arizona, Tucson, Arizona, USA

<sup>m</sup>Washington University, St. Louis, Missouri, USA

<sup>n</sup>University of Wisconsin—Madison, Madison, Wisconsin, USA

<sup>o</sup>University of Maryland, College Park, Maryland, USA

<sup>p</sup>University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

<sup>q</sup>University of North Carolina, Chapel Hill, North Carolina, USA

<sup>r</sup>Oregon Health and Science University, Beaverton, Oregon, USA

<sup>s</sup>SUNY Upstate Medical University, Syracuse, New York, USA

<sup>t</sup>University of Virginia, Charlottesville, Virginia, USA

<sup>u</sup>Tufts University, Boston, Massachusetts, USA

<sup>v</sup>University of Michigan, Ann Arbor, Michigan, USA

**Copyright** © 2023 Goodrum et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Felicia Goodrum, fgoodrum@arizona.edu.

The authors declare a conflict of interest.

Sana Biotechnology has licensed intellectual property of D.B. and Washington University in St. Louis. Gilead Sciences has licensed intellectual property of D.B. and Stanford University. D.B. is a co-founder of Clade Therapeutics. D.B. previously served on an advisory panel for GlaxoSmithKline. D.B. and The University of Arizona hold a patent on SARS-CoV-2 serological assays. K.C. is a special government employee and a member of the Office of AIDS Research Advisory Committee. T.S.D.: Board of Directors, Burroughs Wellcome Fund; Editor, *Annual Review of Virology*. D.D. is on the scientific advisory board of Theriva. W.P.D. receives support from Moderna. M. F. receives funding from National Institute of Allergy and Infectious Diseases (NIAID), Biomedical Advanced Research and Development Authority (BARDA), Defense Advanced Research Projects Agency (DARPA), Gates Foundation, Aikido Pharma,

(Continued on next page)

- <sup>w</sup>University of California, San Diego, La Jolla, California, USA
- <sup>x</sup>Johns Hopkins University, Baltimore, Maryland, USA
- <sup>y</sup>Harvard Medical School, Boston, Massachusetts, USA
- <sup>z</sup>University of Pittsburgh, Pittsburgh, Pennsylvania, USA
- <sup>aa</sup>Western University, London, Ontario, Canada
- <sup>bb</sup>Yale University, New Haven, Connecticut, USA
- <sup>cc</sup>University of Kentucky, Lexington, Kentucky, USA
- <sup>dd</sup>University of Utah, Salt Lake City, Utah, USA
- <sup>ee</sup>Princeton University, Princeton, New Jersey, USA
- <sup>ff</sup>Icahn School of Medicine at Mount Sinai, New York, New York, USA
- <sup>gg</sup>Montana State University, Bozeman, Montana, USA
- <sup>hh</sup>University of Toronto, Toronto, Ontario, Canada
- <sup>ii</sup>Florida Research and Innovation Center, Port Saint Lucie, Florida, USA
- <sup>jj</sup>Loyola University, Maywood, Illinois, USA
- <sup>kk</sup>University of California, Berkeley, Berkeley, California, USA
- <sup>ll</sup>Columbia University, New York, New York, USA
- <sup>mm</sup>University of Washington, Seattle, Washington, USA
- <sup>nn</sup>Duke University, Durham, North Carolina, USA
- <sup>oo</sup>Arizona State University, Tempe, Arizona, USA
- <sup>pp</sup>MRC-University of Glasgow, Glasgow, United Kingdom
- <sup>qq</sup>Baylor University, Houston, Texas, USA
- <sup>rr</sup>Louisiana State University, Shreveport, Louisiana, USA
- <sup>ss</sup>Ulm University, Ulm, Germany
- <sup>tt</sup>University of Massachusetts, Worcester, Massachusetts, USA
- <sup>uu</sup>Northwestern University, Chicago, Illinois, USA
- <sup>vv</sup>University of Minnesota, Minneapolis, Minnesota, USA
- <sup>ww</sup>Dartmouth College, Lebanon, New Hampshire, USA
- <sup>xx</sup>The Ohio State University, Columbus, Ohio, USA
- <sup>yy</sup>University of Iowa, Iowa City, Iowa, USA
- <sup>zz</sup>Iowa State University, Ames, Iowa, USA
- <sup>aaa</sup>New York University, New York, New York, USA
- <sup>bbb</sup>University of Rochester, Rochester, New York, USA
- <sup>ccc</sup>Emory University, Atlanta, Georgia, USA
- <sup>ddd</sup>Cleveland Clinic, Cleveland, Ohio, USA
- <sup>eee</sup>Wake Forest University, Winston-Salem, North Carolina, USA
- <sup>fff</sup>University of Southern California, Los Angeles, California, USA
- <sup>ggg</sup>Wayne State University, Detroit, Michigan, USA
- <sup>hhh</sup>University of Texas, Dallas, Texas, USA
- <sup>iii</sup>Georgia State University, Atlanta, Georgia, USA
- <sup>jjj</sup>Michigan State University, East Lansing, Michigan, USA
- <sup>kkk</sup>The Rockefeller University, New York, New York, USA
- <sup>lll</sup>St. Jude Children's Research Hospital, Memphis, Tennessee, USA
- <sup>mmm</sup>University of California, Irvine, Irvine, California, USA
- <sup>nnn</sup>University of Colorado, Boulder, Colorado, USA
- <sup>ooo</sup>The Peter Doherty Institute, Melbourne, Victoria, Australia
- <sup>ppp</sup>The Pennsylvania State University, University Park, Pennsylvania, USA
- <sup>qqq</sup>Medical College of Wisconsin, Milwaukee, Wisconsin, USA
- <sup>rrr</sup>University of Georgia, Athens, Georgia, USA
- <sup>sss</sup>A\*STAR Infectious Diseases Labs, Singapore
- <sup>ttt</sup>Kansas State University, Manhattan, Kansas, USA
- <sup>uuu</sup>University of Cambridge, Cambridge, United Kingdom
- <sup>vvv</sup>University of Pennsylvania, Philadelphia, Pennsylvania, USA
- <sup>www</sup>University of Connecticut, Farmington, Connecticut, USA
- <sup>xxx</sup>Monash University, Clayton, Victoria, Australia

*(Continued from previous page)*

Emergent, Astrazeneca, Novavax, Regeneron, and the CDC, outside the submitted work. M. F. received royalties/licenses from Aikido Pharma for antiviral drug patent licensing, consulting fees from Aikido Pharma, Observatory Group, for consulting for COVID-19, and participation on Scientific Advisory Board for Aikido Pharma, outside the submitted work. K.F. has substantial financial interest in Vir Biotechnology, Inc., and is a co-founder of the company. K.F. is co-inventor of patents licensed to Vir and receives compensation for consulting for Vir. The potential conflicts of interest have been reviewed and managed by OHSU. D.G. is on the HPV Global Advisory Board for Merck. The A.G.-S. laboratory has received research support from GSK, Pfizer, Senhwa Biosciences, Kenall Manufacturing, Blade Therapeutics, Avimex, Johnson & Johnson, Dynavax, 7Hills Pharma, Pharmamar, ImmunityBio, Accurius, Nanocomposix, Hexamer, N-fold LLC, Model Medicines, Atea Pharma, Applied Biological Laboratories and Merck. A.G.-S. has consulting agreements for the following companies involving cash and/or stock: Castlevax, Amovir, Vivaldi Biosciences, Contrafect, 7Hills Pharma, Avimex, Pagoda, Accurius, Esperovax, Farmak, Applied Biological Laboratories, Pharmamar, CureLab Oncology, CureLab Veterinary, Synairgen and Pfizer. A.G.-S. has been an invited speaker in meeting events organized by Seqirus, Janssen, Abbott and Astrazeneca. A.G.-S. is inventor on patents and patent applications on the use of antivirals and vaccines for the treatment and prevention of virus infections and cancer, owned by the Icahn School of Medicine at Mount Sinai, New York. B.A.G. is on the scientific advisory board for IMUNON. A.L.G. reports central testing contracts from Pfizer, Janssen, Novavax, Sanofi, Abbott, Hologic, Cepheid, and Quidel, and research support from Gilead. E.E.H. is co-founder of Thyreos Inc. E.C.H. has carried out research work in collaboration with AstraZeneca and received honoraria for consultancy work for the Center for Open Science and Seqirus. M.J.I. consults for Gilead Sciences and Via Nova Therapeutics. D.M.K. is a consultant, Replay Bio. T.K. receives funding from Moderna related to CMV. A.S.L. is a paid member of steering committee for clinical trial of baloxavir (Roche). A.C.L. is an inventor on the patent Influenza Vaccines and Uses Thereof, owned by the Icahn School of Medicine at Mount Sinai. G.M. is co-founder of a biotech company devoted to oncolytic virotherapy. W.A.M. is co-inventor of a pending patent application on improved mRNA vaccines. A. Moscona is scientific founder of Thylacine Biotherapeutics, a startup company for antiviral therapeutics. J.A.N., Vir Biotechnology. W.A.O., uncompensated member, Moderna Scientific Advisory Board. D.A.O., Defense Threat Reduction Agency. L.M.S. is a virologist performing research on potential antivirals. L.M.S. is the president-elect of the International Society for Antiviral Research, the membership of which includes people working in companies working on antivirals. L.M.S. has performed paid and non-paid consultancies with private companies developing antivirals or

*(Continued on next page)*

**ABSTRACT** Viruses have brought humanity many challenges: respiratory infection, cancer, neurological impairment and immunosuppression to name a few. Virology research over the last 60+ years has responded to reduce this disease burden with vaccines and antivirals. Despite this long history, the COVID-19 pandemic has brought unprecedented attention to the field of virology. Some of this attention is focused on concern about the safe conduct of research with human pathogens. A small but vocal group of individuals has seized upon these concerns – conflating legitimate questions about safely conducting virus-related research with uncertainties over the origins of SARS-CoV-2. The result has fueled public confusion and, in many instances, ill-informed condemnation of virology. With this article, we seek to promote a return to rational discourse. We explain the use of gain-of-function approaches in science, discuss the possible origins of SARS-CoV-2 and outline current regulatory structures that provide oversight for virological research in the United States. By offering our expertise, we – a broad group of working virologists – seek to aid policy makers in navigating these controversial issues. Balanced, evidence-based discourse is essential to addressing public concern while maintaining and expanding much-needed research in virology.

**KEYWORDS** COVID-19, Coronavirus, DURC, Gain of function, SARS-CoV-2, biosafety, influenza, pandemic, vaccines, zoonosis

Just 30,000 nucleotides of single-stranded RNA, neatly packaged as a coronavirus, brought the world to its knees socially, economically, ethically, and morally during the COVID-19 pandemic. COVID-19 has cast a harsh light on the many cracks, fissures and disparities in our public health system, and the inability to broadly come together to face a colossal crisis and focus on the needs of the most vulnerable. However, scientists worked together and responded to the threat with impressive speed, drawing on critical previous research on coronaviruses and other viral systems. Virologists, immunologists and microbiologists from around the globe collaborated together with scientists from allied disciplines, such as infectious diseases and epidemiology. They confronted the virus through research to understand its pathogenesis and transmission, through surveillance to track the emergence of variants, and through the development of rapid tests, vaccines, antivirals and monoclonal antibodies. The SARS-CoV-2 pandemic would have claimed a substantially larger number of lives and caused more economic disruption were it not for this unprecedented collaborative scientific response. Nevertheless, the SARS-CoV-2 pandemic has also brought virology under the microscope with concerns about safety of virology research and the uncertainties around the origins of SARS-CoV-2. Here we provide an evidence-based discourse to address key issues.

**Virology research under scrutiny.** Congress has a constitutional mandate to provide oversight to federally funded research. As a new Congress convenes in the United States, there is an opportunity for oversight hearings related to research in virology and the virology community stands ready to partner with Congress and lend our expertise. Our hope is that these hearings will highlight the enormous contributions of virology, including gain-of-function experiments, to human health (Table 1). However, we fear that some may use any such hearings to discredit virology and virologists and – whether intentional or not – add fuel to an anti-science, fear-based movement. Should such hearings lead to Congress legislating restrictions on scientific research, the outcome could impede our ability to predict, prepare, and respond to emerging viral threats. An equally devastating outcome would be to sow even more public distrust in science, which would limit our ability to confront viruses in general and increase the human burden from viral diseases.

**The origin of SARS-CoV-2.** A major point of contention in discussions of the COVID-19 pandemic has been the origin of SARS-CoV-2, with two major camps arguing that the virus either originated from animal-to-human transmission (zoonosis) or by a laboratory leak (1–3). Most virologists have been open-minded about the possible origins of SARS-CoV-2 and have formed opinions based on the best available evidence, as is done for all scientific questions (4). While each of these possibilities is plausible and has been investigated, currently the zoonosis hypothesis has the strongest supporting evidence (5–8). Zoonosis involves

(Continued from previous page)

virucidals. L.M.S. is a founder member of a start-up company that had an interest in antivirals. L.M.S. has received funding to work on antivirals. S.S. is Contract Consultant for Curevo Vaccines. J.W.S. consults for the Federal Trade Commission on matters related to COVID-19. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays which list V.S. as co-inventor. G.A.S. is President of Thyreos, Inc. M.S.S. serves an advisory role with Moderna and Ocugen. S.K.W. is co-founder of startup Quercus Molecular Design. C.M.R.: Founder, Apath LLC; Advisor, SAB, and Research Support, Vir Biotechnology; Research support, GSK; SAB Invisishield; SAB Arbutus. C.R.B. is consultant for Ocular Services On Demand (OSOD) located in Madison, WI, and a collaborator with Amebagone a small biotech company in Madison, Wisconsin. C.R.B. has no financial interest in Amebagone and is not owner or part owner of OSOD. All other authors report no conflicts of interest.

**Ed. Note:** This commentary is being published by the following ASM journals: *Journal of Virology*, *mBio*, and *mSphere*.

[This article was published on 26 January 2023 with the incorrect given name for Matthew Daugherty, omissions in the affiliations, and small errors in the text and footnotes. Corrections were made in the version posted on 7 February 2023. An additional footnote correction was made in the current version, posted 28 February 2023.]

The views expressed in this article do not necessarily reflect the views of the journal or of ASM.

**Published** 26 January 2023

**TABLE 1** Human viral diseases for which virology research has delivered vaccines and antiviral drugs

Disease	Vaccine	Antiviral
Adenovirus	Yes	No
AIDS	No	Yes
Cervical and Head/neck Cancer	Yes	No
COVID-19	Yes	Yes
Ebola virus	Yes	Yes
Japanese encephalitis	Yes	No
Hepatitis A	Yes	No
Hepatitis B	Yes	Yes
Hepatitis C	No	Yes
Herpes (HSV and CMV)	No	Yes
Influenza	Yes	Yes
Measles	Yes	No
Mpox	Yes	Yes
Mumps	Yes	No
Polio	Yes	No
Rabies	Yes	No
Respiratory syncytial virus	No	Yes
Rotavirus	Yes	No
Rubella	Yes	No
Smallpox	Yes	Yes
Tick borne encephalitis	Yes	No
Yellow fever	Yes	No
Varicella and zoster	Yes	Yes

transmission of the virus as a consequence of close proximity between humans and wild animals, a scenario that has occurred repeatedly over time, leading to the emergence of many viruses, including Ebola virus, other coronaviruses, influenza A virus, mpox virus, and others (9–11). The lab-origin hypothesis suggests an accident at best or nefarious actors at the worst. At this time and based on the available data, there is no compelling evidence to support either of these lab-origin scenarios. It is important that scientists, the public, and public figures follow the evidence and limit speculation that can become fodder for misinformation and conspiracy theories. For example, on January 1, 2023, *USA Today* published an article discussing where the next pandemic could originate and disproportionately emphasized risk from manmade threats and lab accidents while minimizing the fact that most pandemics are zoonoses and never mentioning how virological research could mitigate risk (12). Unfounded accusations of a lab leak event or nefarious research in Chinese laboratories will hasten the deterioration of important partnerships between the US and China that are critical for early detection and preparedness for seasonal influenza and future pandemics.

**Gain-of-function research.** Despite the paucity of evidence for a laboratory-origin of SARS-CoV-2, discussion of this possibility has driven a second controversy to the forefront of science policy discussion: the use of gain-of-function approaches in virology. Although the phrase ‘gain-of-function’ is very problematic and inexact, it is commonly used, and we will use it here cautioning all its limitations (13). The source of concern in this area is that changing a virus to add new functionality may yield a dangerous pathogen. It is important to understand, however, that gain-of-function approaches incorporate a large proportion of *all* research because they are a powerful genetic tool in the laboratory. These include the development of cancer therapeutics, bacterial strategies for bioremediation, and the engineering of drought- or pest-resistant crops (Table 2). For example, some oncolytic viruses used to treat cancer mediate their effects because, using gain-of-function approaches, they have been endowed with new properties that kill tumors. At least two FDA-approved products resulted from providing viruses with new functions (Table 2). Gain-of-function research with pathogens of pandemic potential established that avian influenza viruses have the capacity to acquire mammalian transmissibility and that bat-associated coronaviruses posed a danger to humans years before COVID-19 (Table 2).

**TABLE 2** Examples of useful gain-of-function experiments

Goal/result	Microbe	Gain-of-Function	Reference
Insect control	Baculovirus	Scorpion neurotoxin	(18)
Solid tumor therapy	Vaccinia	GM-CSF Expression	(19) <sup>a</sup>
Melanoma therapy	Herpes Simplex	GM-CSF Expression	(20)
COVID-19 vaccine	Adenovirus type 26	Expression of SARS-CoV-2 spike protein	(21) <sup>a</sup>
Repair Cardiac pacemaker	Adenovirus	Expression of sinoatrial node transcription factor	(22)
Treatment of bacterial infectious diseases	Bacteriophages	Expression of various payloads to enhance activity	(23)
Treating Citrus tree greening disease	Citrus tristeza virus	Spinach Defensin expression	(24)
Enhanced Lithium Batteries	E4 and M13 bacteriophage	Modified coat protein for carbon nanotube and cation binding	(25)
Rabbit control through immunocontraception	Myxoma virus	Expression of rabbit zona pellucida glycoproteins	(26) <sup>b</sup>
Mouse control through immunocontraception	Ectromelia	Expression of mouse zona pellucida glycoproteins	(27) <sup>b</sup>
Faster computers	M13 bacteriophage	Increased electrical conductance	(28)
Established that H5N1 has capacity for mammalian transmissibility	H5N1	Mutations leading to mammalian transmission	(29, 30)
Established danger from bat SARS-like coronavirus to humans	SARS-CoV	Bat coronavirus spike protein	(31)
Drought and salt resistance in plants	<i>Arabidopsis</i>	Over expression of vacuolar H <sup>+</sup> -ATPase	(32)
Resistance to dengue virus to reduce transmission	Mosquitoes	Transgenic expression of antibody to dengue virus	(33)
Resistance to freezing	Many species from plant to animals	Expression of anti-freeze proteins	(34)
Increase nitrogen fixation to reduce fertilizer need	<i>Klebsiella variicola</i>	122-fold increase in nitrogen fixation genes	(35)
Develop a new vaccine against cryptococcosis	<i>Cryptococcus neoformans</i>	Expression of gamma- interferon	(36)
Hormones for human therapy (e.g. insulin)	<i>E. coli</i>	Synthesis of human hormone (e.g. insulin)	(37)
Enzymes for food prep such as pectinases for improved juice production	Yeast species	Enzyme expression for industrial use	(38)
CAR T cells	Lentivirus	Cancer immunotherapy	(39)
Dengue vaccine	Dengue/yellow fever virus	Recombinant DNA technology replaces genetic sequences in the yellow fever vaccine with dengue virus sequences	(40) <sup>b</sup>

<sup>a</sup>FDA approved product.

<sup>b</sup>Experiments done in viruses that classify as pathogens with pandemic potential.

Despite these clear benefits, a narrative has developed suggesting that gain-of-function research is synonymous with high-risk or nefarious activity to engineer or enhance pandemic pathogens. In truth, gain-of-function research is a valuable experimental approach that virologists use to address essential questions. Virologists do not take their work lightly and thoughtfully propose experiments to address essential questions. Virologists do not operate in isolation to judge the risks of experiments: layers of regulation are in place such that risks are considered by individuals with diverse perspectives and expertise (Fig. 1). The vast majority of virology experiments could not enhance pandemic potential (referred to in the United States as gain-of-function research-of-concern). Those rare experiments that could are currently subject to stringent oversight through the U.S. Government under programs known as dual-use- research-of-concern (DURC) (14) and potential-pandemic-pathogens-care-and-oversight (P3CO) (15) (Fig. 2), and also by the vast majority of international publishers, including the American Society for Microbiology (ASM) and the Public Library of Science (PLOS) (Fig. 3). There are clearly experiments where the risks outweigh the benefits, and it is important that mechanisms exist to prevent such experiments. However, in many cases, gain-of-function research-of-concern can very clearly advance pandemic preparedness and the development of vaccines and antivirals. These tangible benefits often far outweigh the theoretical risks posed by modified viruses. Thus, it is important that oversight mechanisms faithfully consider both risks and benefits of these types of studies. It is equally important that the mechanisms used to provide oversight of gain-of-function research-of-concern be focused on research that is indeed of concern. Identifying research of concern is complex but it is critical that safeguards be both thoughtfully designed and implemented to avoid suppressing innovation in a field essential to mitigating infectious disease threats and with the potential to transform health.

## Federal and Institutional Regulations that Cover the Majority of Microbiology Research

### Federal Regulations

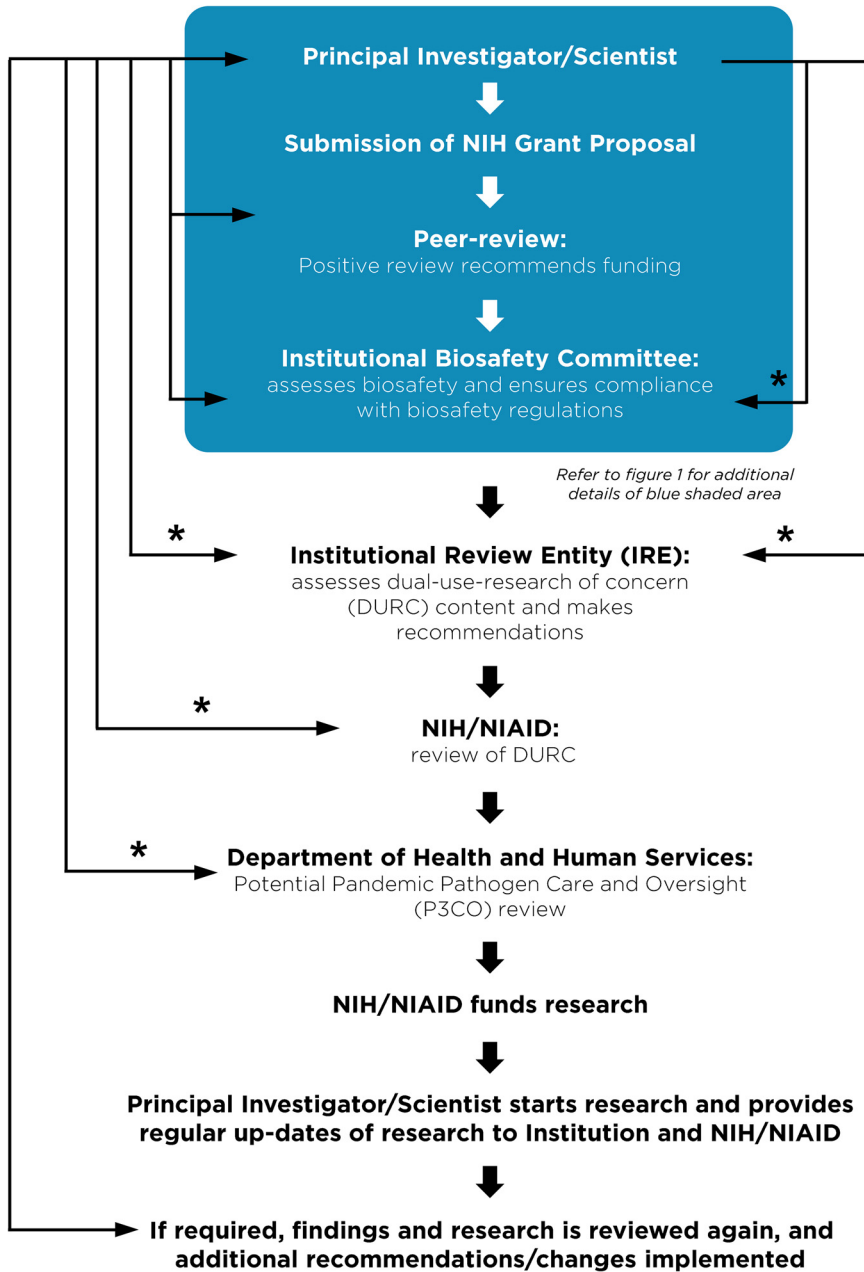
1. **HHS / CDC - Biosafety for Microbiological and Biomedical Laboratories**
  - Sets guidelines and biosafety levels (BSL 1, 2, 2 enhanced, 3, and 4) for research on pathogens
2. **HHS / NIH -Guidelines for Research involving recombinant DNA or Synthetic Nucleic Acid Molecules (84 FR 17858 )**
  - Sets the guidelines for institutional Biosafety Committees (IBC)
  - Requires 2 community members (not employed by the Institution) be voting members of the committee.
3. **USDA APHIS -**
  - Registration of research with animal or plant pathogens (e.g. avian influenza viruses)
  - Permit requires on-site inspections every 3 years and annual renewals

### Institutional Regulations

1. **Environmental Health and Safety Office (EHSO)**
  - Oversees annual laboratory inspections.
  - Sets biosafety training requirements for research staff.
  - Maintains records of biosafety inspections and trainings.
2. **Institutional Biosafety Committee (IBC)**
  - Reviews the procedures and experimental details of any research involving genetic manipulation of viruses and bacteria.
  - Makes recommendations regarding biosafety level and practices to ensure safety.
  - Notifies the NIH of research not conducted in accordance of 84 FR 17858 guidelines.
  - Engages the institutional Review Entity (IRE), which has institutional oversight over dual use research of concern, as appropriate.
  - Committee includes principal investigators, biosafety officers (from the EHSO), 2 community members and others with relevant expertise.
  - Approval of a protocol by the IBC is required before the initiation of any research.

**FIG 1** Federal and institutional regulations. A brief breakdown of current U.S. Department of Health and Human Services regulations on microbiology research and the implementation of federal requirements by individual institutions, through various committees and processes. Important additional oversight that is not shown includes that provided by occupational health services to ensure safety of research personnel, and federal and institutional regulation of research involving vertebrate animals or human subjects, which are overseen by Institutional Animal Care and Use Committees (IACUC; animal research) and Institutional Review Boards (IRB; human subjects research).

**Existing regulation of virology research in the United States.** Without qualification, appropriate precautions should be taken to minimize laboratory accidents or the unjustified engineering of pathogens with enhanced pandemic potential. This is a shared goal for scientists and regulators. Virological research in the United States is subject to federal regulation through the Department of Health and Human Services and the U.S. Department of Agriculture for work involving human, animal, and plant pathogens. Federal policies are



**FIG 2** Current regulations surrounding funding, monitoring and approval of gain-of-function research of concern. A flow chart describing the steps a principal investigator must follow prior to receiving funding or initiating research considered dual-use-research-of-concern (DURC) on pathogens of pandemic potential (P3). The blue shaded box corresponds to the general practices that all investigators carry out at the institutional level described in Fig. 1. Research with the potential to enhance the properties of pathogen is referred for additional oversight. Asterisks (\*) indicate an iterative process typically involving 1–3 reviews and revisions.

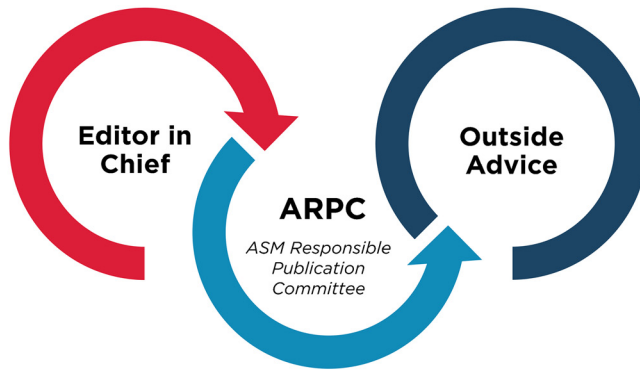
guided by the Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines (16) published by the Centers for Disease Control and Prevention (CDC) and implemented by Institutional Biosafety Committees (IBCs) that manage the safety and practice of laboratory research at the local level (Fig. 1). The National Institutes of Health (NIH) established guidelines for the creation of IBCs in 1976 and updates these guidelines periodically. While each institution may have additional specific policies, the NIH requires that IBCs approve of research involving genetic manipulation of pathogens and that members of the local community are engaged through their inclusion on IBCs. As noted above,

## ASM Journals DURC/GOF/P3 Process

### SCREEN

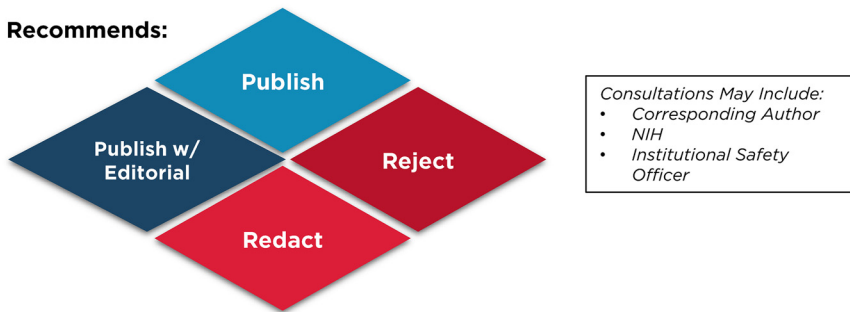


### DISCUSS



### DECIDE

**ARPC Recommends:**



**FIG 3** Publishing process for articles involving gain-of-function research-of-concern or with pathogens of pandemic potential at ASM journals. Articles in the GOF/DURC/P3 category are flagged by multiple layers at the screening stage. This process will initiate discussion among the scientific editor in chief and other subject matter experts and lead to a publication decision after careful consideration of risks and benefits.

further oversight for research involving viruses with pandemic potential exists at multiple levels, including federal funding agencies (e.g., Department of Health and Human Services, Department of Defense), institutions (e.g., colleges, universities, and research institutes), and most accredited publishers of scientific research (Fig. 2 and 3) (17). It is important that we work to ensure appropriate and consistent implementation of these existing safeguards. We should also seek to develop international partnerships to ensure appropriate oversight is in place worldwide. While policy and infrastructure should frequently be reassessed with the goal of updating and improving biosafety and biosecurity, policy should not be changed rashly and without consideration of potential unintended consequences. Given the scientific complexities involved, such decisions should not be legislated. They should instead be made after full consideration by scientists with the relevant expertise – with appropriate oversight from Congress.



**Conclusion.** As we enter the fourth year of the COVID-19 pandemic, it is clear that the scientific establishment has been the most effective shield protecting humanity from this calamity through the delivery of rapid tests, vaccines, antibody therapies, and small molecule antivirals. Millions are alive today that would otherwise be dead thanks to society's investment in creating and maintaining a vibrant scientific enterprise. In the debate on further restrictions around gain-of-function research and new regulations on virology in general, it is important to appreciate the existing framework of regulations at the federal and institutional levels. It is also critical to recognize that our ability to respond rapidly to emerging viral threats is dependent on our ability to apply the tools of modern biology to viruses. Regulations that are redundant with current practice or overly cumbersome will lead to unwarranted constraints on pandemic preparation and response and could leave humanity more vulnerable to future disease outbreaks. Gain-of-function research has been an extremely valuable tool in the development of vaccines and antivirals (Table 2). It already has layers of regulations and checks in place to ensure that potentially unsafe research is immediately reported (Fig. 2). It is critical that policy makers, virologists, and biosafety experts work together to ensure that research is conducted safely, with the common goal of reducing the burden of disease caused by viruses.

### ACKNOWLEDGMENTS

The opinions expressed reflect those of the authors, and not necessarily the opinions of ASM, the authors' institutions, or funding agencies. We acknowledge Johnny Chang for his assistance with graphics.

No funding was used to support this commentary, but we wish to disclose funding from agencies received by authors. JCA None WJA NIH DCA NIH JDB NIH BWB CIHR, NSERC LB Associazione Italiana per la Ricerca sul Cancro, ICGEB SBM NIH DB NIH, Private Foundation DBM Private Foundation DCB NIH, Private Foundation ACB NIH SB None CRB NIH, NSF, Private Foundation AJB USDA, Biotechnology and Biological Sciences Research Council (BBSRC), UK CBB NIH, DOD CEC NIH SKC NIH PC NIH AC NIH GCC NIH ARC NIH, Private Foundation JMC NIH KC NIH BD NIH MDD NIH, Burroughs Wellcome Investigators in the Pathogenesis of Infectious Disease Program KD None JAD NIH, Private Foundation TSD NIH, Private Foundation, Heinz Endowments JDD Canadian Institutes of Health Research and Natural Sciences and Engineering Council of Canada DD NIH RRD NIH, USDA, Private Foundation WPD NIH, Zoetis LLC, Moderna, Coalition for Epidemics Preparedness Initiatives (CEPI) RED NIH NCE NIH, Howard Hughes Medical Institute ME NIH LWE None BAF NSF AFS NIH MLF NSF, USDA, Private Foundation LF Canadian Institutes of Health Research (CIHR) MBF NIH, Novavax, Astrazeneca, Pfizer, BARDA, Eli Lilly KF NIH, DOD, Bill and Melinda Gates Foundation MUG NIH MMG NIH, Private Foundation TG NIH DG NIH, Private Foundation AG-S NIH, DOD, Private Foundation APG NIH BAG NIH, Howard Hughes Medical Institute SPG NIH, Private Foundation FG NIH ALG None MHH NIH EH NIH NSH NIH, DOD, Private Foundation MTH NIH EEH NIH, Private Foundation BGH NIH, NSF SMH NIH ECH UK Medical Research Council, UK Medical Research Council JMH NIH MJI NIH, Via Nova Therapeutics WTJ NIH RFK NIH JPK NIH SMK NIH FK German research foundation (DFG), European research council (ERC) DMK NIH TK NIH, Moderna ML NIH LL NIH SSL NIH RAL NIH ASL NIH, Burroughs Wellcome Fund, Flu Lab, CDC BL NIH, NSF, Private Foundation DAL NIH SL NIH, Private donor's fund, Ohio State. startup fund RML NIH CBL NIH ACL NIH, Private Foundation MAL NIH JML NIH BM NIH GM NIH MTM NIH, DHS AMehle NIH, Private Foundation WAM NIH, Plant Sciences Institute, Iowa State University IM NIH CM NIH NJM NIH Amoscona NIH BCM NIH KM NIH JCM NIH EAM NIH MHN NIH JAN NIH CJN None JZN NIH CMO None AO NIH WAO NIH, Bill & Melinda Gates Foundation DAO NIH, Private Foundation JHJO NIH JSP NIH CRP NIH, Private Foundation AP NIH, Private Foundation, CDC PEP NIH JKP NIH, Private Foundation RKP NIH, DOD, Gilead Sciences, Enanta Pharmaceuticals SJP None JGP NIH DP NIH, Private Foundation MQM NIH, Private Foundation RR NIH CMR NIH, Private Foundation RJR NIH CJR NIH RS-G NIH MS NIH LMS NIH, Cornell University, Private donors, Fast Grants SS None JWS NIH SSC NIH, Private Foundation BLS NIH TS NIH, DOD GS NIH, Private Foundation VS NIH, Private Foundation GAS NIH, USDA JGS NIH KRS NIH MLS start-up funds from the University of

Florida KS Australian government, NHRMC WIS NIH MSS NIH TCS NIH, USDA AWT NIH VLT NIH, Private Foundation BT NIH, DOD, Private Foundation SAT NIH SMT NIH ZT NIH KVD NIH, Private Foundation MV None NAW NIH DW NIH MPW Medical Research Council, UK (MR/W025647/1); UKRI mpox research consortium (BB/X011143/1) JBW NIH MDW NIH SKW NIH EAW NIH, Private Foundation SPJW NIH, Private Foundation, USAID, Vir Biotechnology BRGW Private Foundation CEW NIH, University of Michigan SWW NIH ADY NIH.

## REFERENCES

- Cohen J. 2022. Studies bolster pandemic origin in Wuhan animal market. *Science* 375:946–947. <https://doi.org/10.1126/science.adb1760>.
- Maxmen A. 2022. Wuhan market was epicentre of pandemic's start, studies suggest. *Nature* 603:15–16. <https://doi.org/10.1038/d41586-022-00584-8>.
- Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. 2020. The proximal origin of SARS-CoV-2. *Nat Med* 26:450–452. <https://doi.org/10.1038/s41591-020-0820-9>.
- Casadevall A, Weiss SR, Imperiale MJ. 2021. Can Science Help Resolve the Controversy on the Origins of the SARS-CoV-2 Pandemic? *mBio* 12:e0194821. <https://doi.org/10.1128/mBio.01948-21>.
- Worobey M, Levy JI, Malpica Serrano L, Crits-Christoph A, Pekar JE, Goldstein SA, Rasmussen AL, Kraemer MUG, Newman C, Koopmans MPG, Suchard MA, Wertheim JO, Lemey P, Robertson DL, Garry RF, Holmes EC, Rambaut A, Andersen KG. 2022. The Huanan Seafood Wholesale Market in Wuhan was the early epicenter of the COVID-19 pandemic. *Science* 377:951–959. <https://doi.org/10.1126/science.abp8715>.
- Relman DA. 2020. Opinion: To stop the next pandemic, we need to unravel the origins of COVID-19. *Proc Natl Acad Sci U S A* 117:29246–29248. <https://doi.org/10.1073/pnas.2021133117>.
- Holmes EC, Goldstein SA, Rasmussen AL, Robertson DL, Crits-Christoph A, Wertheim JO, Anthony SJ, Barclay WS, Boni MF, Doherty PC, Farrar J, Geoghegan JL, Jiang X, Leibowitz JL, Neil SJD, Skern T, Weiss SR, Worobey M, Andersen KG, Garry RF, Rambaut A. 2021. The origins of SARS-CoV-2: A critical review. *Cell* 184:4848–4856. <https://doi.org/10.1016/j.cell.2021.08.017>.
- Garry RF. 2022. The evidence remains clear: SARS-CoV-2 emerged via the wildlife trade. *Proc Natl Acad Sci U S A* 119:e2214427119. <https://doi.org/10.1073/pnas.2214427119>.
- Plowright RK, Parrish CR, McCallum H, Hudson PJ, Ko AI, Graham AL, Lloyd-Smith JO. 2017. Pathways to zoonotic spillover. *Nat Rev Microbiol* 15:502–510. <https://doi.org/10.1038/nrmicro.2017.45>.
- Albery GF, Becker DJ, Brierley L, Brook CE, Christofferson RC, Cohen LE, Dallas TA, Eskew EA, Fagre A, Farrell MJ, Glennon E, Guth S, Joseph MB, Mollentze N, Neely BA, Poisot T, Rasmussen AL, Ryan SJ, Seifert S, Sjodin AR, Sorrell EM, Carlson CJ. 2021. The science of the host-virus network. *Nat Microbiol* 6:1483–1492. <https://doi.org/10.1038/s41564-021-00999-5>.
- Taubenberger JK, Kash JC. 2010. Influenza virus evolution, host adaptation, and pandemic formation. *Cell Host Microbe* 7:440–451. <https://doi.org/10.1016/j.chom.2010.05.009>.
- Weintraub K. 2023. As COVID turns 3, experts worry where the next pandemic will come from — and if we will be ready, *In USA Today*. <https://www.usatoday.com/story/news/health/2023/01/01/covid-anniversary-next-pandemic-expert-concern/10847848002/>.
- Dance A. 2021. The shifting sands of 'gain-of-function' research. *Nature* 598:554–557. <https://doi.org/10.1038/d41586-021-02903-x>.
- USDoHaH S. 2021. Dual Use Research of Concern 2021. <https://www.phe.gov/s3/dualuse/Pages/default.aspx>. Accessed.
- USDoHaH S. 2021. Department of Health and Human Services (HHS) Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens <https://www.phe.gov/s3/dualuse/Pages/p3co.aspx>. Accessed.
- (ed). 2020. Biosafety in Microbiological and Biomedical Laboratories (BMBL). Centers for Disease Control and Prevention and U.S. National Institutes of Health. <https://www.cdc.gov/labs/BMBL.html>. Accessed.
- Casadevall A, Dermody TS, Imperiale MJ, Sandri-Goldin RM, Shenk T. 2015. Dual-Use Research of Concern (DURC) Review at American Society for Microbiology Journals. *mBio* 6:e01236. <https://doi.org/10.1128/mBio.01236-15>.
- Stewart LM, Hirst M, López Ferber M, Merryweather AT, Cayley PJ, Possee RD. 1991. Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. *Nature* 352:85–88. <https://doi.org/10.1038/352085a0>.
- Kim JH, Oh JY, Park BH, Lee DE, Kim JS, Park HE, Roh MS, Je JE, Yoon JH, Thorne SH, Kim D, Hwang TH. 2006. Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF. *Mol Ther* 14:361–370. <https://doi.org/10.1016/j.jymthe.2006.05.008>.
- Bommareddy PK, Patel A, Hossain S, Kaufman HL. 2017. Talimogene Laherparepvec (T-VEC) and Other Oncolytic Viruses for the Treatment of Melanoma. *Am J Clin Dermatol* 18:1–15. <https://doi.org/10.1007/s40257-016-0238-9>.
- Gray JJ, Moughon S, Wang C, Schueler-Furman O, Kuhlman B, Rohl CA, Baker D. 2003. Protein-protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. *J Mol Biol* 331:281–299. [https://doi.org/10.1016/s0022-2836\(03\)00670-3](https://doi.org/10.1016/s0022-2836(03)00670-3).
- Kapoor N, Liang W, Marbán E, Cho HC. 2013. Direct conversion of quiescent cardiomyocytes to pacemaker cells by expression of Tbx18. *Nat Biotechnol* 31:54–62. <https://doi.org/10.1038/nbt.2465>.
- Meile S, Du J, Dunne M, Kilcher S, Loessner MJ. 2022. Engineering therapeutic phages for enhanced antibacterial efficacy. *Curr Opin Virol* 52:182–191. <https://doi.org/10.1016/j.coviro.2021.12.003>.
- Ghosh D, Motghare M, Gowda S. 2018. Citrus greening: overview of the most severe disease of citrus. *Adv Agric Res Technol J* 2:83–100.
- Lee YJ, Yi H, Kim WJ, Kang K, Yun DS, Strano MS, Ceder G, Belcher AM. 2009. Fabricating genetically engineered high-power lithium-ion batteries using multiple virus genes. *Science* 324:1051–1055. <https://doi.org/10.1126/science.1171541>.
- Mackenzie SM, McLaughlin EA, Perkins HD, French N, Sutherland T, Jackson RJ, Inglis B, Müller WJ, van Leeuwen BH, Robinson AJ, Kerr PJ. 2006. Immun contraceptive effects on female rabbits infected with recombinant myxoma virus expressing rabbit ZP2 or ZP3. *Biol Reprod* 74:511–521. <https://doi.org/10.1095/biolreprod.105.046268>.
- Jackson RJ, Maguire DJ, Hinds LA, Ramshaw IA. 1998. Infertility in mice induced by a recombinant ectromelia virus expressing mouse zona pellucida glycoprotein 3. *Biol Reprod* 58:152–159. <https://doi.org/10.1095/biolreprod58.1.152>.
- Loke DK, Clausen GJ, Ohmura JF, Chong T-C, Belcher AM. 2018. Biological-templating of a segregating binary alloy for nanowire-like phase-change materials and memory. *ACS Appl Nano Mater* 1:6556–6562. <https://doi.org/10.1021/acsnano.8b01508>.
- Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de WE, Munster VJ, Sorrell EM, Bestebroer TM, Burke DF, Smith DJ, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. 2012. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336:1534–1541. <https://doi.org/10.1126/science.1213362>.
- Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K, Zhong G, Hanson A, Katsura H, Watanabe S, Li C, Kawakami E, Yamada S, Kiso M, Suzuki Y, Maher EA, Neumann G, Kawaoka Y. 2012. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486:420–428. <https://doi.org/10.1038/nature10831>.
- Menachery VD, Yount BL, Jr, Debbink K, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Ge XY, Donaldson EF, Randell SH, Lanzavecchia A, Marasco WA, Shi ZL, Baric RS. 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* 21:1508–1513. <https://doi.org/10.1038/nm.3985>.
- Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR. 2001. Drought- and salt-tolerant plants result from overexpression of the AVP1 H<sup>+</sup>-pump. *Proc Natl Acad Sci U S A* 98:11444–11449. <https://doi.org/10.1073/pnas.191389398>.
- Buchman A, Gamez S, Li M, Antoshechkin I, Li HH, Wang HW, Chen CH, Klein MJ, Duchemin JB, Crowe JE, Jr, Paradkar PN, Akbari OS. 2020. Broad dengue neutralization in mosquitoes expressing an engineered antibody. *PLoS Pathog* 16:e1008103. <https://doi.org/10.1371/journal.ppat.1008103>.

34. Naing AH, Kim CK. 2019. A brief review of applications of antifreeze proteins in cryopreservation and metabolic genetic engineering. *3 Biotech* 9: 329. <https://doi.org/10.1007/s13205-019-1861-y>.
35. Wen A, Havens KL, Bloch SE, Shah N, Higgins DA, Davis-Richardson AG, Sharon J, Rezaei F, Mohiti-Asli M, Johnson A, Abud G, Ane JM, Maeda J, Infante V, Gottlieb SS, Lorigan JG, Williams L, Horton A, McKellar M, Soriano D, Caron Z, Elzinga H, Graham A, Clark R, Mak SM, Stupin L, Robinson A, Hubbard N, Broglie R, Tamsir A, Temme K. 2021. Enabling Biological Nitrogen Fixation for Cereal Crops in Fertilized Fields. *ACS Synth Biol* 10:3264–3277. <https://doi.org/10.1021/acssynbio.1c00049>.
36. Wormley FL, Jr, Perfect JR, Steele C, Cox GM. 2007. Protection against cryptococcosis by using a murine gamma interferon-producing *Cryptococcus neoformans* strain. *Infect Immun* 75:1453–1462. <https://doi.org/10.1128/IAI.00274-06>.
37. Riggs AD. 2021. Making, Cloning, and the Expression of Human Insulin Genes in Bacteria: The Path to Humulin. *Endocr Rev* 42:374–380. <https://doi.org/10.1210/endrev/bnaa029>.
38. Blanco P, Sieiro C, Villa TG. 1999. Production of pectic enzymes in yeasts. *FEMS Microbiol Lett* 175:1–9. <https://doi.org/10.1111/j.1574-6968.1999.tb13595.x>.
39. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. 2018. CAR T cell immunotherapy for human cancer. *Science* 359:1361–1365. <https://doi.org/10.1126/science.aar6711>.
40. Juraska M, Magaret CA, Shao J, Carpp LN, Fiore-Gartland AJ, Benkeser D, Girerd-Chambaz Y, Langevin E, Frago C, Guy B, Jackson N, Duong Thi Hue K, Simmons CP, Edlefsen PT, Gilbert PB. 2018. Viral genetic diversity and protective efficacy of a tetravalent dengue vaccine in two phase 3 trials. *Proc Natl Acad Sci U S A* 115:E8378–E8387. <https://doi.org/10.1073/pnas.1714250115>.