

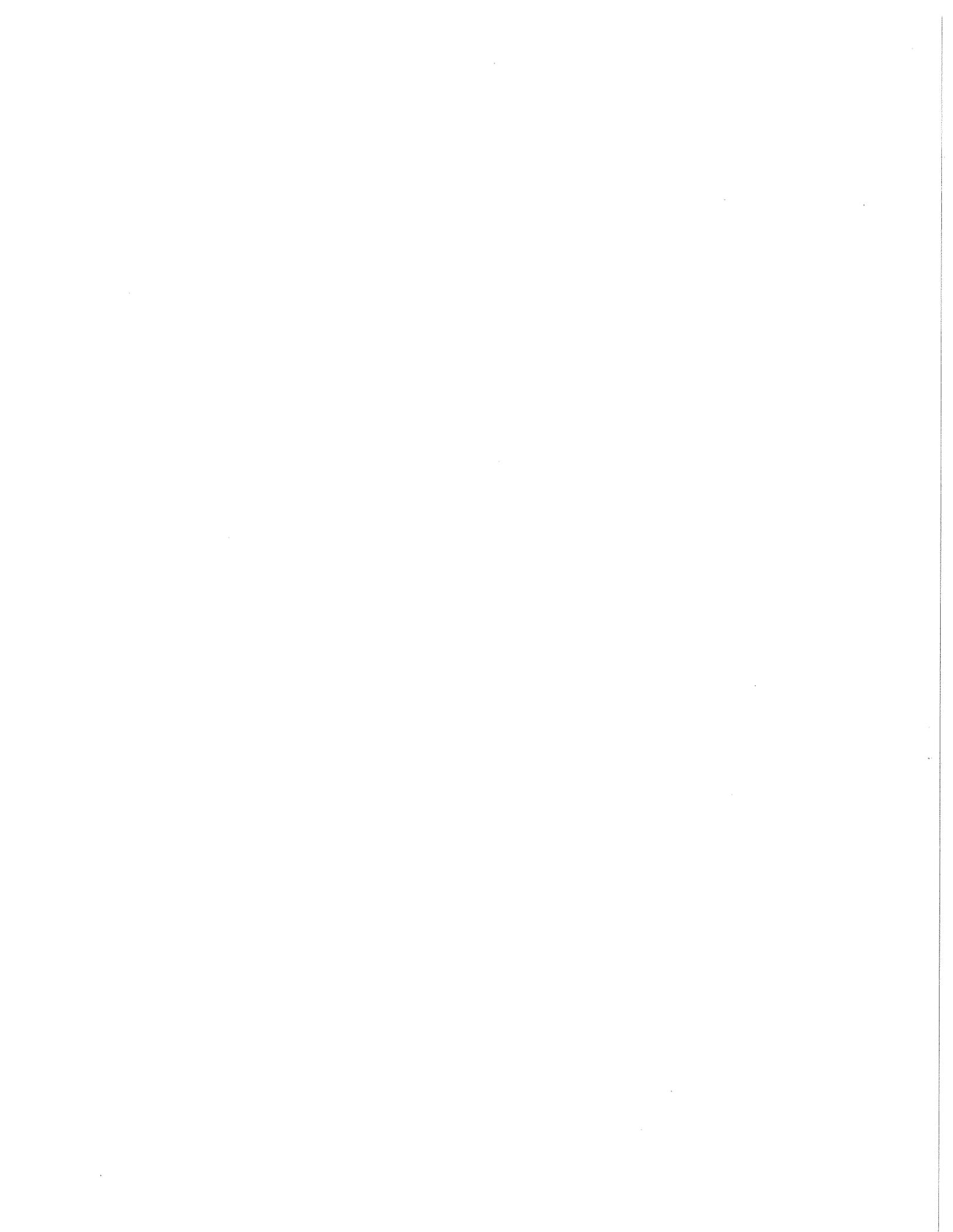
National Institute of Dental and Craniofacial Research

National Advisory Dental and
Craniofacial Research Council

Minutes of Meeting
January 31, 2018

Building 31
Conference Room 6
National Institutes of Health
Bethesda, Maryland

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH



DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF DENTAL AND CRANIOFACIAL RESEARCH

MINUTES OF THE
NATIONAL ADVISORY DENTAL AND CRANIOFACIAL RESEARCH COUNCIL

January 31, 2018

The 217th meeting of the National Advisory Dental and Craniofacial Research Council (NADCRC) was convened on January 31, 2018, at 9:00 a.m., in Building 31, Conference Room 6, National Institutes of Health (NIH), Bethesda, Maryland. The Council met in closed session from 9:00 a.m. to 10:26 a.m. for Council business and consideration of grant applications. The meeting was open to the public from 10:30 a.m. until 11:44 a.m. and, after a lunch break, from 12:45 p.m. until adjournment at 2:25 p.m. Dr. Martha Somerman presided as Chair.

CLOSED SESSION

This portion of the meeting was closed to the public in accordance with the determination that it was concerned with matters exempt from mandatory disclosure under Sections 552b(c)(4) and 552b(c)(6), Title 5, U.S. Code and Section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2).

REVIEW OF APPLICATIONS

Grant Review

The Council considered 242 applications requesting \$57,725,718 in total costs. The Council recommended 217 applications for a total cost of \$51,457,748 (see Attachment II).

OPEN SESSION

Members Present

Dr. Shenda M. Baker
Dr. Yang Chai
Dr. Richard Peters Darveau
Dr. Nisha J. D'Silva
Ms. Tracy Hart
Dr. Daniel Malamud
Dr. Daniel W. McNeil
Dr. Phillip B. Messersmith
Dr. Anne Louise Oaklander
Dr. Sanjay Shete

Dr. Anne C. R. Tanner
Dr. Jane A. Weintraub

Members of the Public

Dr. Seun Ajiboye, Director, Science Policy and Government Affairs, International Association for Dental Research (IADR)/American Association for Dental Research (AADR), Alexandria, VA
Dr. Marcelo W. B. Araujo, Vice President, Science Institute, American Dental Association (ADA), Washington, D.C.
Mr. Omar Contreras, Director of Policy Research, Office of Policy, Research, and Diversity, American Dental Education Association (ADEA), Washington, D.C.
Dr. Floyd E. Dewhirst, Senior Member of Staff, Department of Microbiology, The Forsyth Institute, Cambridge, MA
Dr. Christopher Fox, Executive Director, IADR/AADR, Alexandria, VA
Ms. Lindsey Horan, Assistant Director of Government Affairs, IADR/AADR, Alexandria, VA
Dr. Patrick M. Lloyd, Dean and Professor, College of Dentistry, The Ohio State University, Columbus, OH
Dr. Mircea Podar, Distinguished Scientist and Systems Genetics Group Leader, Oak Ridge National Laboratory, Oak Ridge, TN
Ms. Christina McWilson Thomas, Director for Government Affairs, Advocacy and Government Relations, ADEA, Washington, D.C.

National Institute of Dental and Craniofacial Research

Dr. Martha J. Somerman, Director
Dr. Alicia Dombroski, Executive Secretary, and Director, Division of Extramural Activities (DEA)
Dr. Douglas Sheeley, Deputy Director
Dr. Lillian Shum, Director, Division of Extramural Research (DER)
Dr. Robert Angerer, Scientific Director, Division of Intramural Research (DIR)
Dr. Matthew P. Hoffman, Deputy Scientific Director, DIR
Dr. Nisan Bhattacharya, DEA, Scientific Review Branch (SRB)
Dr. Preethi Chander, DER, Integrative Biology and Infectious Diseases Branch (IBIDB)
Dr. Latarsha Carithers, DEA, SRB
Ms. Vicki Contie, Office of the Director (OD), Office of Communications and Health Education (OCHE), Science Communication and Digital Outreach Branch (SCDOB)
Ms. Michelle Cortes, DER IBIDB
Ms. Mary Cutting, DER, Center for Clinical Research (CCR)
Ms. Mary Daum, OD, OCHE, Health Information and Public Liaison Branch (HIPLB)
Mr. Bret Dean, OD, Office of Administrative Management (OAM), Financial Management Branch (FMB)
Dr. Olga Epifano, DEA
Dr. Catherine Evans, OD, OCHE, SCDOB
Dr. Dena Fischer, DER, CCR
Dr. Leslie Frieden, DEA, Research Training and Career Development Branch (RTCDB)
Dr. Crina Frincu, DEA, SRB

Dr. Gallya Gannot, DER, CCR
Dr. Nicole Garcia-Quijano, OD, OCHE, HIPLB
Mr. Joel Guzman, DER, Translational Genomics Research Branch (TGRB)
Dr. Sue Hamann, OD, Office of Science Policy and Analysis (OSPA)
Ms. April Harrison, DEA, Grants Management Branch (GMB)
Ms. Jeannine Helm, DER
Mr. Gabriel Hidalgo, DEA, GMB
Dr. Jonathan Horsford, OD, OSPA
Dr. Lynn Mertens King, DEA, RTCDB
Dr. Wendy Knosp, OD, OSPA
Dr. Jamie Kugler, Office of the Scientific Director, DIR
Ms. Carol Loose, OAM, FMB
Mr. Orlando Lopez, DER, IBIDB
Ms. Susan Lowenthal, DEA, GMB
Dr. Nadya Lumelsky, DER, IBIDB
Dr. R. Dwayne Lunsford, DER, IBIDB
Ms. Jayne Lura-Brown, DER
Ms. Yasamin Moghadam, DER, CCR
Dr. Marilyn Moore-Hoon, DEA, SRB
Dr. Dawn Morales, DER, Behavioral and Social Sciences Research Branch (BSSRB)
Mr. Paul Newgen, DEA, GMB
Dr. Morgan O'Hayre, OD
Ms. Lisa Peng, OD, Office of Information Technology (OIT)
Mrs. Debbie Pettitt, DEA, GMB
Ms. Ann Poritzky, OD, OCHE, SCDOB
Mr. John Prue, OD, OIT
Dr. Melissa Riddle, DER, BSSRB
Ms. Delores Robinson, DEA
Dr. Steven Scholnick, DER, TGRB
Dr. Yasaman Shirazi, DEA, SRB
Dr. Kathryn Stein, DER, TGRB
Ms. Kathleen Stephan, OD
Mr. Brian Sullivan, OD, OIT
Mr. Joseph Tiano, OD, OSPA
Dr. Yolanda Vallejo-Estrada, DER, IBIDB
Dr. Jessica Walrath, OD, OSPA
Dr. Jason Wan, DER, IBIDB
Dr. S. Chiayeng Wang, DER, IBIDB
Dr. Darien Weatherspoon, DER, CCR
Dr. Gary Zhang, DEA, SRB

Other Federal Employees

Dr. Thomas Hart, Senior Director, ADA Foundation Volpe Research Center (VRC), National Institute of Standards and Technology (NIST), Department of Commerce (DoC), Gaithersburg, MD

Dr. Jeffrey Kim, Principal Investigator, VRC, NIST, DoC, Gaithersburg, MD
Dr. Lu Wang, Division of Genome Sciences, National Human Genome Research Institute
(NHGRI), NIH

I. WELCOME AND INTRODUCTIONS

Dr. Martha Somerman, Director, NIDCR, called the open session of the 217th meeting of the Council to order. She noted that 2018 is the 70th anniversary of NIDCR, which was established in 1948 as the National Institute of Dental Research in response to the caries epidemic. Dr. Somerman commented that while little was understood about microbes in health and disease at that time, microbes have become a prime research topic, and their contribution to oral health and disease is the focus of the current Council meeting.

Dr. Somerman thanked the Council members, staff, and other participants for attending the meeting. She especially thanked Council members whose terms had expired and agreed to continue their service until new members are designated. Dr. Somerman introduced Dr. Douglas Sheeley, who was appointed deputy director of the NIDCR in October 2017. Dr. Sheeley has a long career at the NIH and was previously senior scientific officer and acting chief of the Biomedical Technology Branch at the National Institute of General Medical Sciences. Dr. Somerman invited all guests to introduce themselves.

Dr. Alicia Dombroski, Executive Secretary, and Director, Division of Extramural Activities (DEA), welcomed the Council and all attendees, including those attending via the NIH videocast (<http://videocast.nih.gov>). She also especially welcomed Drs. Anne Louise Oaklander, Dr. Anne C. R. Tanner, and Dr. Jane A. Weintraub for extending their service on the Council during the hiring delay.

II. FUTURE MEETING DATES

May 25, 2018
September 13, 2018
January 23, 2019
May 23, 2019
September 13, 2019

III. APPROVAL OF MINUTES FROM PREVIOUS MEETING

Dr. Alicia Dombroski invited the Council to consider and approve the minutes of the September 15, 2017, Council meeting. The Council unanimously approved the minutes.

IV. ANNUAL REVIEW OF COUNCIL OPERATING PROCEDURES

Dr. Dombroski invited the Council to suggest changes, comment on, or raise questions about the “Operating Procedures of the National Advisory Dental and Craniofacial Research Council,” which was distributed to all Council members. She noted that no changes had been made in the operating procedures this year.

The Council unanimously approved the operating procedures.

V. REPORT OF THE DIRECTOR, NIDCR

Dr. Somerman presented the budget for Fiscal Year (FY) 2017 and various updates. She described the NIDCR portfolio and funding trends; NIDCR funding initiatives; NIH activities under the 21st Century Cures effort and the Opioid Research Initiative; and the status of NIDCR 2030. Dr. Somerman’s written Director’s Report to the Council: January 2018 was provided to the Council members and is available at (<http://www.nidcr.nih.gov>).

NIDCR Budget, Portfolio, and Funding Trends

Dr. Somerman reported that the actual budget for the NIDCR in FY 2017 was \$424,782,000, which is an increase over the FY 2016 budget. Approximately 78 percent (or ~\$331 million) of the total was allocated to the extramural program, 16 percent (or \$66,785 million) to the intramural program, and 6 percent (or \$46,987) for research management and support (RMS). Of the total funds allocated to the intramural program, approximately 38 percent was for central NIH assessments, which included increases for security, information technology, study sections, and maintenance and repairs.

Most of the overall budget (63 percent) was allocated to the extramural program in support of 667 research grants. These included research project grants (RPGs), which accounted for 81 percent of the extramural budget, as well as small business initiatives, research centers, research careers, and other related research. Dr. Somerman reported that 77 percent of the total NIDCR budget was committed to ongoing research (i.e., non-competing) and as the cost of these grants increases, the amount available to support new, competing grants decreases. Support for various RPG mechanisms remained fairly stable in FY 2017. This included support for investigator-initiated R01s (which accounted for 72 percent of all RPG support), cooperative agreements [e.g., for Practice-Based Research Networks (PBRNs), Clinical Centers on Oral Health Disparities, and Genome-Wide Association Studies], and increases in set-aside programs [e.g., the Dental, Oral, and Craniofacial (DOC) Tissue Regeneration Consortium (DOCTRC), Supplements to Advance Research (STAR) awards, and oral health disparities research].

Dr. Somerman reported that the success rate for NIDCR research grant applications in FY 2017 was approximately 18 percent, which is in line with the NIH average, and that the overall decline in NIH success rates since FY 2003 is a concern at the NIH. She noted that in FY 2017, the NIDCR success rate for RPG applications held steady at about 18 percent for R01s and R03s, but declined to less than 10 percent for R21s. The NIDCR has increased the dollar amount of

R21 awards, which support high-risk, high-return research, to foster more applications. Dr. Somerman encouraged early-stage investigators (ESIs) who are seeking research support after their R03s to apply for R01s, rather than R21s, because of the higher success rate of R01 applications.

RFAs, PAs, and Future FOAs

Dr. Somerman highlighted several NIDCR initiatives. In FY 2017, NIDCR continued support for research on HIV and HIV-associated conditions, provided new support for research on biosensors in the oral cavity, and initiated support for implementation science research to improve DOC health. The NIDCR continued support for DOCTRC resource centers and the NIDCR Award for Sustaining Outstanding Achievement in Research (SOAR).

In FY 2018, the NIDCR intends to support research in response to RFAs related to HIV and HIV-associated conditions, neoantigens-based treatments, neuroskeletal biology, and the biology of aging in DOC tissues. The NIDCR also plans to support an NIDCR Dental Specialty and Ph.D. Program (DSPP) and to continue support for SOAR awards. The NIDCR also hopes to fund applications received in response to many important PAs. These include understanding gender differences in treatment effects, immune system responses to medications, diagnostic use of sophisticated imaging systems, and biomarkers of DOC diseases and conditions. In addition, the NIDCR will continue support for R03, K99/00, and K awards for research training and career development. Dr. Somerman encouraged the Council members to communicate the availability of the NIDCR Dual Degree Dentist Scientist Pathway to Independence Award (K99/00) to ESIs.

Dr. Somerman noted the many concepts approved by Council for which the NIDCR will be issuing FOAs. These pertain to HIV/AIDS research, bioinformatics, enamelogenesis, gene-environment interactions, imaging of oral lesions, biological functions underlying oral health disparities, FaceBase 3, and PBRNs.

Appropriations, Legislation, and Transition

Dr. Somerman expressed appreciation to the Congress for the budget increase in FY 2017. She commented that the continued decline in purchasing power since FY 2013 remains a concern. There is as yet no budget for FY 2018 and the NIH is operating under a second continuing resolution through February 8.

Other Updates

NIH Updates. Dr. Somerman reported that Dr. Alex Azar was sworn in on January 29 as Secretary of the U.S. Department of Health and Human Services. On October 17, Dr. Norman E. “Ned” Sharpless became the new director of the National Cancer Institute. Dr. Somerman said that the NIH is pleased with Dr. Azar’s appointment and that Dr. Sharpless is supportive of the NIDCR portfolio in oral cancer. She noted further that four dentists have been serving in the U.S. House of Representatives since 2016.

Dr. Somerman reported that the Congress has authorized \$4.8 billion to the NIH over 10 years for research under the 21st Century Cures effort. The four main areas are: Cancer Moonshot, Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, All of Us Research Program, and Regenerative Medicine Innovation Project. She emphasized that the NIDCR is actively participating in the All of Us program and will sponsor a special workshop, entitled “Precision Medicine Cohort Program,” at the 47th annual meeting of the American Association for Dental Research (AADR), in Ft. Lauderdale, Florida, March 21–24, 2018. Dr. Somerman emphasized that dentistry must be integrated with medicine in this NIH-wide effort, which will link research and patients’ electronic health records, and she encouraged the Council members to communicate the need for oral health practitioners and patients to participate in All of Us. The 21st Century Cures effort is funded by set-aside monies that are appropriated annually by the Congress.

Dr. Somerman also highlighted the NIDCR’s participation in the Regenerative Medicine Innovation Project, a trans-NIH activity coordinated with the Food and Drug Administration (FDA) to utilize adult stem cells, including autologous stem cells, for tissue regeneration. During the first phase of implementation, in FY 2017, \$2.0 million was allocated from 21st Century Cures funding and, along with \$0.7 million from the NIH institutes and centers (ICs) and non-Federal 1:1 matching funds, supported eight competitive supplements to “jump-start” public-private partnership research. For FY 2018, \$10 million has been proposed to fund a Regenerative Medicine Innovation Workshop, to be co-hosted by the NIH and FDA on December 17 and address standardization, regulatory issues, multidisciplinary research, development of new models, and preparation for manufacturing.

Dr. Somerman reported on potential policy changes for the Next Generation Researchers Initiative (NGRI), a component of the 21st Century Cures effort. Five policy goals are to protect ESIs, stabilize career trajectories of successful and productive mid-career investigators, understand and mitigate unintended consequences, implement rigorously vetted and evidence-based policies, and have robust mechanisms for ongoing monitoring and re-evaluation of policies. At its December 17th meeting, the Advisory Council to the Director, NIH, convened an NGRI working group that will advise the NIH leadership on development of a trans-NIH NGRI policy. The working group’s report is expected in June 2018.

Dr. Somerman commented on the NIH Opioid Research Initiative. She noted that the emphasis is on public-private partnerships in research to end the opioid crisis. The three focus areas are pain management, opioid addiction treatment, and overdose reversal.

NIDCR 2030. Dr. Somerman noted that NIDCR 2030: Envisioning the Future, Together is a strategic action plan that is separate from the NIDCR Strategic Plan 2014–2019 and is intended to be bold and innovative. Launched in March 2017 at the annual AADR meeting, it has five themes: oral health + overall health, precision health, autotherapies, oral biodevices, and workforce diversity. The NIDCR Ideascale website attracted more than 225 ideas on these themes from more than 660 registered users, who comprised researchers, patients, dental health professionals, and industry representatives. Dr. Somerman reported that Dr. Morgan O’Hayre, who is spearheading this effort, and staff “curated” all this information and identified specific

actions for each area. The NIDCR has now entered the implementation phase of NIDCR 2030, which began with a trans-NIH symposium and workshop, entitled “Autotherapies: Enhancing Our Innate Healing Capacity,” held at the NIH on January 25–26. Important themes at the workshop included the need for biological research to understand the many signaling pathways and the response of different tissues in autotherapy. Topics specific to oral health included mechanisms of pain, microbiomes, precision medicine targets, rare diseases, and workforce diversity to enrich the research environment. The NIDCR is now preparing its next strategic plan for release in 2020.

In closing, Dr. Somerman encouraged everyone to “connect with us” through www.nidcr.nih.gov.

VI. CONCEPT CLEARANCE

Dr. Dombroski, Director, DEA, stated that the NIDCR is required to present the purpose, scope, and objectives of proposed concepts for research initiatives to the Council in a public forum for the Council’s review, discussion, and approval and for public comment. Concepts that are approved by the Council are published on the NIDCR website. The NIDCR presented one concept, as follows.

Enabling Technologies to Accelerate Development of Oral Biodevices

Dr. Orlando Lopez, Director, Dental Materials and Biomaterials Program, Integrative Biology and Infectious Diseases Branch (IBIDB), Division of Extramural Research (DER), presented a concept for research on enabling technologies to accelerate development of oral biodevices. Dr. Lopez noted that oral biodevices is one of the five themes of NIDCR 2030 and that upon the Council’s approval, the concept will be posted on the NIDCR Ideascale for comment. The goals of this concept are (i) to advance technical development and clinical translation of innovative oral biodevices and (ii) to use the mouth as a portal for biodevices to assess oral and overall health in real time, diagnose diseases, and deliver local and systemic treatments.

Dr. Lopez noted that the current state of the science and advances in core technologies allow development of simple, fast, reliable, and robust oral devices; that oral biodevices can provide real-time, continuous, and noninvasive monitoring of oral/dental and overall health; and that integration of oral biodevices and drug delivery systems can improve clinical treatment and compliance of patients. This research will entail development of safe and effective solutions to overcome challenges imposed by the oral environment, such as pH and temperature levels, extreme and repetitive masticatory forces, and acceptance by patients. Dr. Lopez highlighted specific areas of research interest that include, but are not limited to, the following: validation of sensing and interfacing approaches for building safe and effective oral biodevices; optimization of oral biodevices’ biocompatibility, shelf-life, and adhesion to wet intraoral tissues with minimal biofouling effects; development of oral biodevices for biomarker detection to empower precision

medicine-based approaches; and development of sensitive and specific oral biodevices to measure clinically relevant outputs and endpoints across medical treatments.

The Council's discussants, Dr. Shenda M. Baker, Dr. Daniel Malamud, and Dr. Phillip B. Messersmith, strongly favored the concept. They noted that the proposed research is both exciting and compelling and that the concept is well articulated and would generate much interest in the research community. They commented on the current lack of tools to assess and track both local and systemic effects of diseases and treatments. Dr. Malamud noted that applicants will need to consider various issues and approaches to this research. Drs. Baker and Messersmith highlighted the multidisciplinary nature of the research and encouraged broad outreach across the research community, which could include convening a symposium to engage researchers (e.g., electrical engineers) who are largely outside of the NIDCR's purview.

In response to the Council's questions, Dr. Lopez noted that the proposed concept builds on an earlier, 2016 FOA supporting research on development of biosensors. The present concept encourages development of biodevices that offers integrated systems for diagnosis, treatment, and disease management and incorporates other parameters, such as patients' acceptance. It would "push the envelope" to address a broad array of biomarkers and design constraints (e.g., force, hormones, temperature, pH), include related research on biomarkers in the nasal cavity (e.g., nasal obstruction, viral infections, allergen exposure), incorporate low-power electronics, and address cybersecurity concerns regarding secure wireless transmission of patients' information.

The Council unanimously approved the concept.

VII. SPECIAL SESSION UNCULTIVATED BACTERIA OF THE ORAL MICROBIOTA

Dr. R. Dwayne Lunsford, Director, Microbiology Program, IBIDB, DER, introduced the special session on uncultivated bacteria of the oral microbiota. He noted that with completion of the first phase of the NIH Common Fund Human Microbiome Project (HMP), researchers have entered "the candy store" of a post-microbiome era in which they can integrate datasets of microbiology properties to study host-microbiome interactions in health and disease. [This research is the focus of the second phase of the HMP (i.e., the integrated HMP, or iHMP).]

Dr. Lunsford defined "uncultivated" bacteria as those that are viable organisms that cannot be cultivated using (current) standard techniques. He noted that much of the microbial universe is viewed as "dark matter," as up to 50 percent of micro-organisms cannot be cultivated and most microbial divisions do not have a single cultivated representative. The question arises, "What are they doing?" Are they bystanders, pathogens, and/or modulators of virulence? What is their role in oral health and disease? Do they have a role in biofilm formation, metabolic cooperativity, or persistence?

Dr. Lunsford noted that NIDCR-supported investigators are uniquely positioned to answer these questions, owing to the ease of sampling in the oral cavity; their decades of research in microbial physiology and ecology, resulting in a rich legacy of phylogenetic studies; and the availability now of the Human Oral Microbiome Database (HOMD). The NIDCR has taken the lead in stimulating this research. In October 2012, the Council reviewed and approved an NIDCR concept to encourage research on the oral microbiota; in May 2013, the NIDCR issued an RFA for research on Innovative Approaches and Technologies for Examining the Uncultivable Bacteria of the Oral Microbiome (RFA-DE-14-003); and in August 2014, the NIDCR funded two awards. Dr. Lunsford noted that the NIDCR is the only IC supporting research specifically on the domestication and basic biology of uncultivability. Two other ICs are, respectively, supporting research on uncultivated flora to define biosynthetic pathways for drug development and exploring secondary metabolites of host–microbe interactions in the gut.

Dr. Lunsford introduced the two principal investigators funded under RFA-DE-14-003, who presented updates on the status of their projects for the Council. Their presentations are summarized below in sections VIII and IX.

VIII. CULTIVATION, NATURE, ECOLOGY, AND PATHOGENICITY OF THE UNCULTIVABLE ORAL MICROBIOME

Dr. Floyd E. Dewhirst, Senior Member of Staff, Department of Microbiology, The Forsyth Institute, Cambridge, Massachusetts, discussed the definitions of uncultivable bacteria, the cultivability status of the 769 taxa in the electronic HOMD (eHOMD), reasons for uncultivability, and the change in conceptual frameworks that is allowing for progress.

Dr. Dewhirst suggested that for the NIDCR to achieve the HMP goal, which is “...to fully understand the human microbiome,” it must gain a complete understanding of all bacteria (cultivated and uncultivated) in the mouth. He noted that although only a small proportion of bacteria have been cultured, most microbiologists don’t use the term uncultivable and, instead, hold the view that all bacteria can be cultivated. Of the 769 oral and nasal bacteria in eHOMD, 57 percent (440) have been cultivated and named; 17 percent (100) have been cultivated, but are unnamed; and 30 percent have not been cultivated. Among all 769 taxa, the genomes of 482 (63 percent) have been sequenced, and among the 540 cultured taxa, 482 (~ 90 percent) have been sequenced.

Dr. Dewhirst noted that difficulties in cultivating oral bacteria may arise from various causes or factors such as temperature, pH, oxygen levels; carbon, nitrogen, and sulfur sources; auxotrophy; domestication; and microbial dark matter. He described his research to counter these difficulties and elaborated on auxotrophy as perhaps the major cause of uncultivability. Auxotrophy relates to organisms in the mouth, such as amino, fatty, and nucleic acids, lipids, and vitamins—many of which are in yeast—as well as siderophores, bacterial cell membrane components, and still-unknown factors. Possible solutions for growing bacteria with unknown auxotrophies include growing bacteria in consortia, providing “bacterial extract” to the medium, and cross-streaking with a helper bacterial strain from yeast. Dr. Dewhirst noted that with

addition of a helper strain, for example, his research team was able to grow *Tannerella sp.*, a clone of the 286 taxa; with N-acetylmuramic acid, from the breakdown of peptidoglycan of neighboring bacteria, they grew *Tannerella forsythia*; and with the siderophore Desferricoprogen, they grew *Prevotella sp.*, a clone of the 376 taxa. He also said the team was able to grow *Fretibacterium fastidiosum* by using re-streaking to reduce the complexity of its domestication over time.

Dr. Dewhirst noted that cultivation of bacteria in Candidate Phyla Radiation (CPR), which had not been possible previously, was recently accomplished by growing the bacteria in consortia, rather than reducing the consortia to a single bacterium. Cultivation of the first CPR organism, TM7 (*Saccharibacteria*), a parasite, on *Actinomyces* cells was reported in 2015 by researchers at the University of California at Los Angeles (UCLA), and, since then, UCLA and Forsyth investigators have isolated 10 TM7 strains capable of growing on several different host bacteria (e.g., *Propionibacterium propionicum*). He noted that the TM7 CPR organisms are hardy and, similar to parasites, are of small genome size and can pass through 0.2 μM filters to then be inoculated into other, host bacteria. He further noted that the dark-matter environment of parasites is integral to isolation and cultivation of TM7 organisms and that the same situation may prevail for all CPR bacteria.

Dr. Dewhirst suggested that oral microbiologists' success in culturing oral bacteria may be a result of a shift in conceptual framework, from Koch's approach (i.e., medical microbiology) to Winogradsky's approach (i.e., environmental microbiology). By shifting the research paradigm from a focus on members of a community or consortia to a focus on pathogens and cultivation of individual bacteria in pure culture, researchers have been able to culture TM7 and other, "uncultivable" bacteria. Dr. Dewhirst commended the NIDCR for leading the research on the nature of uncultivability.

In discussion, Dr. Dewhirst noted that TM7 bacteria are elevated in periodontal diseases and that some "uncultivable" bacteria may be susceptible to antibiotics. He also noted that slight genetic mutations may occur with repeat passages during cultivation of bacteria over time and that comparison of their genetic sequences before and after domestication is needed.

IX. CULTURING OF THE UNCULTURED: REVERSE GENOMICS AND MULTI-SPECIES CONSORTIA IN ORAL HEALTH AND DISEASE

Dr. Mircea Podar, Distinguished Scientist and Systems Genetics Group Leader, Oak Ridge National Laboratory, Oak Ridge, Tennessee, described his research in microbiome genetics, which he is conducting in collaboration with geneticists at Ohio State University (OSU). He agreed with Dr. Dewhirst that all bacteria are potentially cultivable. He suggested, however, that there will always be bacteria that will remain unknown, despite the growing number of microbiologists, because there are so many of them and they live in constant fear of being isolated and like to play hide and seek. Still, they are not ultimately uncultivable.

Dr. Podar said that much work has been done in traditional microbiology and that OSU researchers recently reported cultivating a novel organism using this approach. Increasingly, though, microbiologists are using “reverse-genomic” methodology to culture “unculturable” bacteria, and much work is being done with this powerful approach to identify, isolate, and characterize the human oral microbiome and its role in health and disease. With this approach, scientists have been able to perform single-cell genomics and metagenomics. However, making biological inferences from the findings remains difficult. Dr. Podar noted that “we are not there yet” and approximately one-third of HOMD bacteria remain uncultured and/or unnamed. The new “tree of life” includes many bacteria phyla in which individual representatives have not been cultivated and, in two phyla, no representatives have even been identified. And, while researchers have identified a number of microbes involved in oral health and disease (gingivitis and periodontitis), the list is not complete—some bacteria in defined clusters have been identified and named, some are uncultivated, and some are seen frequently and may serve as markers of disease.

Dr. Podar focused on the value and importance of studying microbes in their environment when trying to cultivate a microbial organism. He noted that the interspecies interactions among microbes that may be involved in health and disease include competition, syntrophy, cross-feeding, and symbiosis (as with TM7). The approach used by his research team to study microbes of the human oral cavity includes isolation of single cells or combinatorial populations; study of their interactions, using flow cytometry, cell sorting, and nanoporous microfluidic systems; and optimization of the system. Dr. Podar elaborated on the use of this approach, some of the findings gained, and future directions. He noted, for example, that studies of multiple single-cell genomes of *Desulfobulbus* sp. HOTO41 provided insight into the functions of other, uncultured bacteria in the oral cavity and showed that both *Desulfobulbus* HOTO41 and *Desulfobulbus oralis* were enriched with *Fusobacterium nucleatum*. Further studies are under way to elucidate the factor(s) making the growth of these bacteria possible.

Additional research on *D. oralis*, the first of four host-associated *Desulfobulbus* microbes to be cultured, included physiological testing, gene sequencing, and metabolic reconstruction. This research showed that, in comparison with the many free-living *Desulfobulbus*, *D. oralis* is the smallest microbe of the genus and both loses and gains properties in adaptation to the host. Two interesting findings were that, during host adaptation, *D. oralis* loses its mercury methylation capability, which would be a complicating factor with dental amalgams, and that *D. oralis* can elicit a pro-inflammatory response in oral epithelial cells. Dr. Podar also described research on microbial syntrophy, which led to identification of *Methanobravibacter oralis*, a bacteria that the team is now using in syntrophic enrichment to isolate and characterize previously uncultured bacteria.

Describing research on SR1, a widely distributed candidate bacterial phylum for which there are no cultured representatives as yet, Dr. Podar noted that his team was able to isolate the bacteria and, with flow cytometry, to enrich it sufficiently for sequencing, or cultivation. Through a sequencing study, the team uncovered a novel, ongoing process of codon reassignment, which the researchers are exploring in different protein environments. Similar work is being done to understand the recoding of free-living GNO2 bacteria. Dr. Podar said that

the meaning, mechanism, and utilization of genome recoding in the human microbiota are not known and it is not clear whether the feature is ancestral or derived. He noted that both the SR1 and GNO2 genomes have been characterized and both require cross-feeding or direct association with a host, but how they “make a living” is not yet known. In other studies, the research team is looking at the diversity of the oral TM7 bacteria.

In conclusion, Dr. Podar noted that the research team is screening and sequencing hundreds of microbial cultures developed by mixing cells under different conditions with various growth media and then performing high-throughput cultivation and characterization. The hope is to assemble the findings into a network analysis to identify and statistically discriminate species’ associations (friends and foes) from co-cultures. He emphasized that, in the end, it is the evolution of microbes with respect to host specificity that is important. The conclusion, or “end story,” is that for every microbe in the human species there is a counterpart in dogs or cats and that the origins of the microbiota and the continuing exchange of microbes among species is relevant to understanding and cultivating them and may play a role in health and disease. With this perspective, Dr. Podar’s team is also exploring emerging pathogens, health and disease determinants, and interspecies interaction and culturability in samples from humans and from wolves (antecedents of dogs) in Yellowstone National Park.

X. DISCUSSION

In response to questions, Dr. Podar noted that his team has several hundred cultures and much data related to dental caries and periodontitis, but that the researchers are working with random cells and it is too early to see emerging patterns with respect to oral phenotypes. He also noted that lateral transfer of microbes does occur between humans and other species (e.g., dogs and monkeys). He said that his team has found 20–30 uncultivated bacteria and that the challenge now is to isolate them. He noted the limitations of proteonomics and the need for new, good methods to fractionate samples in order to find missing factors. Both he and Dr. Dewhirst said they are pursuing the use of agarose bead assays to study oral microbes.

Dr. Dombroski thanked both Dr. Dewhirst and Dr. Podar for their presentations. Dr. Somerman thanked the Council members, all attendees and virtual participants, speakers and visitors, and staff for attending the meeting.

XI. ADJOURNMENT

The meeting was adjourned at 2:25 p.m. on January 31, 2018.

CERTIFICATION

I hereby certify that the foregoing minutes are accurate and complete.

 4/24/18

Dr. Martha J. Somerman
Chairperson
National Advisory Dental and
Craniofacial Research Council



Dr. Alicia Dombroski
Executive Secretary
National Advisory Dental and
Craniofacial Research Council

ATTACHMENTS

- I. Roster of Council Members
- II. Table of Council Actions