Carcinogenicity Health Effects Innovation Program

Presenters: Dr. Amy Wang, Integrative Health Assessments Branch, and Dr. Arun Pandiri, Comparative and Molecular Pathogenesis Branch, NIEHS/DNTP

Program Management Team: Warren Casey, Dori Germolec, Alison Harrill, Arun Pandiri, Erik Tokar, and Amy Wang

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Problem Statement

Environmental exposures represent a significant source of cancer risk in the United States and worldwide. The financial impact of cancer on the U.S. economy is significant (~5% of the GDP) and widespread (affecting approximately one in three Americans) and exacerbates socioeconomic and health care inequalities. Cancer prevention efforts, which can be focused on disproportionately affected groups, present important opportunities to protect public health while reducing the negative economic effects of this family of diseases. Mitigating cancer risk by managing exposure to environmental agents of concern is one such opportunity. However, despite enormous gains made over the past 50 years in understanding the pathobiology of human cancers, we currently lack the means to efficiently and effectively identify many agents of concern and accurately characterize the risk(s) they may pose to public health. Establishing such a capability would enable the development of cancer prevention efforts at many different levels (e.g., legislative, corporate, and individual).

Objectives

The Division of the National Toxicology Program (DNTP) is committed to protecting public health and reducing cancer risk by establishing a state-of-the-art translational testing and reporting system that uses disease-based knowledge to efficiently identify and characterize environmental cancer hazards and provide actionable information to all stakeholders in a timely manner. The Carcinogenicity Health Effects Innovation (Carci HEI) Program is structured around the following objectives and subobjectives. Brief descriptions of representative projects and activities for each are presented below.

1. Collaborate with internal and external stakeholders to define, develop, and establish confidence in a new paradigm that will use contemporary knowledge of cancer biology to evaluate and characterize the contribution of environmental exposures to the etiology of human cancers.

   The Carci HEI program will use existing methods and expertise within DNTP (including the 2-year rodent bioassay) while also incorporating new in silico, in vitro, and in vivo approaches into an integrated framework for characterizing the carcinogenic risk posed by environmental substances. The development and validation of these approaches will be guided by the principles outlined in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)'s "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" to help ensure acceptance of these approaches by federal agencies, international regulators, industry stakeholders, and the public. The Carci HEI program will comprise several projects with interrelated and interconnected areas of focus. Examples include:

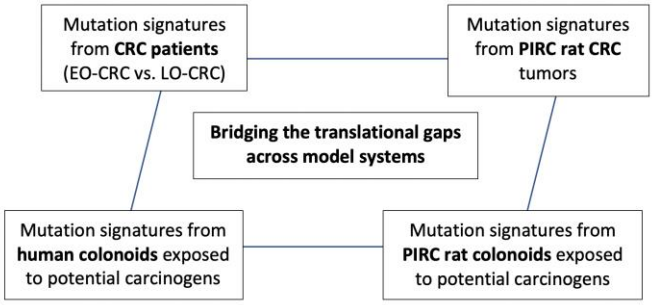
1.1. Develop and evaluate new technologies and approaches with broad translational relevance for evaluating genetic and epigenetic alterations that contribute to the initiation and promotion of tumorigenesis.

- Collaborate with Ramazzini Institute, Italy, and Wellcome Sanger Institute, United Kingdom, to sequence the genomes of chemical-induced rodent tumors from Ramazzini Institute's tissue archives to identify and characterize mutational signatures.
- Collaborate with TwinStrand Biosciences, USA, and Wellcome Sanger Institute to evaluate mutation signatures and cancer-driver genes using whole genome/exome sequencing analysis in archived rodent tissues resulting from exposures to various nongenotoxic carcinogens. Another major goal of this collaboration is to develop a screening panel to identify mutations in cancer-driver genes in nontumor rodent tissues, circulating cell-free DNA (ccfDNA) from short-term (3–6 month) animal studies and from in vitro exposures to evaluate potential cancer hazards. This screening panel will be based on error-corrected duplex sequencing, a new methodology that improves the accuracy of mutation detection ~10,000-fold compared with existing next-generation sequencing methods. This remarkable quantitative sensitivity allows detection of ultra-rare mutations in tissue/cell cultures that are phenotypically normal at earlier time points, well short of those historically used for assessing phenotypic and genetic alterations. The results will be used for prediction of carcinogenicity hazards and may obviate the need to run 2-year rodent cancer bioassays.
- Develop and validate in vitro systems, such as organoids and microphysiological systems incorporating stem cells, which may overcome challenges related to the lack of metabolic competence and the inability to sustain complex cultures over the periods of time needed to detect processes related to tumor pathogenesis.
- Evaluate in vitro systems and associated data aligned to the Key Characteristics of Carcinogens (KCCs) to determine their utility for characterizing and predicting cancer hazards.

1.2. Develop, evaluate, and facilitate the adoption of assays and approaches that use knowledge of human cancer genetics to assess the ability of chemicals to induce tissue-specific cancers in humans.

We will initially prioritize resources for projects related to early-onset colorectal cancer (EO-CRC), which shows an increasing burden of disease in specific human populations and may potentially be associated with environmental exposures. Complementary translational approaches are being developed for identifying substances that may contribute to EO-CRC. Examples include:

- Analyze previously published whole genome and whole-exome sequencing data to identify mutation signatures unique to early- or late-onset CRC, as these may help to understand the underlying mechanisms that can be linked to potential environmental exposures.
- Compare mutation signatures and molecular pathways in human colorectal cancers with those from short-term animal studies in Polyposis in the Rat Colon (PIRC) rats and in vitro colon organoids (colonoids) derived from PIRC rats and humans (induced pluripotent stem cells and embryonic stem cells).



1.3. Develop a dedicated resource for querying and exporting data from NTP cancer studies and other relevant datasets.

With over 600 Technical Reports published, the National Toxicology Program houses the world's largest collection of publicly available data from 2-year rodent cancer bioassays and other associated studies (e.g., dose range-finding studies, 13-week subchronic studies, genetic toxicity studies, toxicokinetic studies). While much of this data are available through the DNTP's Chemical Effects in Biological Systems (CEBS) database, extracting information of interest would require multiple searches and a detailed knowledge of DNTP study design and reporting parameters, followed by additional steps to integrate the disparate data types. To facilitate and encourage the use of computational approaches for characterizing carcinogenic hazards, a purpose-built platform meeting FAIR principles (findability, accessibility, interoperability, and reusability) is being developed that will contain information such as individual animal histopathology, incidence statistics for neoplastic and nonneoplastic lesions, clinical chemistry, and genetic toxicity data. The database will be part of NICEATM's Integrated Chemical Environment (ICE),[1] allowing the integration of bioassay data with other sources of curated data, such as Tox21 high-throughput screening assays, as well as Quantitative Structure Activity Relationship (QSAR) and In vitro to In Vivo Extrapolation (IVIVE) models.

2. Engage, inform, and educate stakeholders on the appropriate interpretation and use of cancer hazard information produced in the new testing and assessment paradigm described above via our communication plan.

The U.S. National Center for Education Statistics defines science literacy as "the knowledge and understanding of scientific concepts and processes required for personal decision-making, participation in civic and cultural affairs, and economic productivity." Traditional approaches to science and health education are no match for modern communication channels (i.e., social media) in which disinformation is pervasive and highly effective, and inaccurate claims regarding the origins of cancer and "carcinogens" are all too common. Although many DNTP stakeholders are well qualified to objectively assess scientific findings from complex studies, misinterpretation by legislators or the public could ultimately result in deleterious decisions for public health and the economy. Developing and implementing a modern education and communication strategy to effectively convey and contextualize information generated as part of the Carci HEI program will therefore be critical to help ensure the appropriate and impactful use of the findings. We recognize that effective outreach and communication is one of our greatest challenges and will require a great

[1] ICE: Integrated Chemical Environment. National Toxicology Program. https://ntp.niehs.nih.gov/go/niceatm-ice

deal of input from scientific communication experts and a wide range of new and existing stakeholders.

Rationale

Public Health Context

Approximately one in three people will be diagnosed with cancer at some point in their lifetime. Despite tremendous progress in screening and treatment efforts, cancer is still the second most common cause of death, and the leading health concern of the American public. In addition to the obvious detrimental effect cancer has on human health, its economic and societal impact are substantial as well. The cost of cancer care in the United States for 2020 was approximately $206 billion[2] and although the incidence and mortality for many cancer types are decreasing, the prevalence of some cancers is increasing in different demographic groups and the overall economic burden of cancer continues to rise annually. Developing an efficient approach for identifying environmental substances that contribute to the etiology of cancer would enable the mitigation of existing exposures and prevention of such exposures in the future.

Alignment with Mission, Goals, Strategic Pipeline

The Carci HEI program will advance the DNTP mission to improve public health through research, testing, and analysis activities that are translatable, predictive, and timely to inform real-life individual and public health outcomes. DNTP will leverage all necessary resources in developing fit-for-purpose testing strategies using both existing and new approaches to characterize the potential carcinogenic risk posed by environmental exposures. We will accomplish our mission by adhering to DNTP's mission and goals by using enhanced stakeholder engagement, applying innovative science to generate trusted information, and training next-generation translational scientists.

Existing approaches for cancer hazard assessment rely primarily on data from whole animal tests that take years to complete, cost millions of dollars, and provide results that may not be directly relevant to human health (over- or under-predicting hazard). With a recognized history in protecting public health, DNTP is well-positioned to take on this challenge relative to other organizations owing to its extensive experience and expertise on cancer hazard assessment and its goal to lead the transformation of toxicology through innovation.

Stakeholder Interest and Engagement

Steps Taken to Engage Stakeholders

DNTP continues to engage subject matter experts and other stakeholders, building on the long-standing relationships developed over the past four decades administering the cancer testing program. Members of the Carci HEI program have engaged and received feedback from stakeholders at numerous meetings and workshops over the past 18 months regarding the proposed concept, including:

- NTP Board of Scientific Counselors Meeting, June 2019, presentation: *Re-envisioning Carcinogenicity Assessment at NTP*

[2] Cancer Prevalence and Cost of Care Projections. National Cancer Institute. https://costprojections.cancer.gov/

- Genetics and Environmental Mutagenesis Society (GEMS) of North Carolina, October 2019, poster: The Environmental Cancer Prevention Initiative at National Toxicology Program (ECPI@NTP)
- 2019 Triangle Global Health Annual Conference, October 2019, poster: The Environmental Cancer Prevention Initiative at National Toxicology Program (ECPI@NTP)
- SOT CSS Webinar, January 2020, presentation: *Advances in Carcinogenicity Assessment at the DNTP*
- Assessing Carcinogenicity: Hazard Identification, Classification, and Risk Assessment. A Toxicology Forum State-of-the-Science Workshop, December 2020, two presentations:
  - *Opportunities to Improve Cancer Risk Assessment*
  - *Interrogating Cancer for NAMs*

Ongoing and Continuing Interactions

Federal agencies, international organizations, industry groups, nongovernmental organizations, and the public rely on data from the NTP 2-year rodent bioassay to inform public health decisions and all of these groups remain important stakeholders. There are a number of historical, ongoing, and planned activities with stakeholder and partner organizations (e.g., National Cancer Institute (NCI), National Center for Advancing Translational Sciences (NCATS), U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration (FDA), European Chemicals Agency (ECHA), International Agency for Research on Cancer (IARC), Organisation for Economic Co-operation and Development (OECD), International Conference on Harmonization (ICH), academic institutions, nonprofit institutions, industry partners) to develop a fundamental and contemporary understanding of carcinogenesis, which can be applied to meeting the goals and objectives of the Carci HEI program. Examples of some of these interactions are provided below:

Stakeholder	Issue	Role of Stakeholder
Wellcome Sanger Institute **Ramazzini Institute** **TwinStrand Biosciences**	Whole genome sequencing to understand mutation signatures in chemical carcinogenesis	Collaborators
Health and Environmental Sciences Institute (HESI) (Emerging Systems Toxicology for Assessment of Risk Committee)	(a) Develop a biomarker strategy to identify potential for nongenotoxic carcinogenesis via nuclear receptor activation; (b) coordinate best practices for using transcriptomic point of departure studies for internal decision-making	Partner
IARC	Approach for genotoxicity evaluation	Collaborator, user
NCI **National Heart, Lung, and Blood Institute** **Duke U.** **U. of Pittsburgh** **U. of North Carolina at Chapel Hill** **Broad Institute**	Plasma liquid biopsy as a novel biomarker tool to identify driver mutations	Collaborators
MD Anderson Cancer Center	Mutation signatures in early onset versus late-onset colorectal cancers	Collaborator

Stakeholder	Issue	Role of Stakeholder
NC3Rs	Promoting the development and evaluation of complex in vitro systems for evaluating the initiation and promotion of tumorigenesis	Collaborator
EPA **PETA International Science Consortium** **Syngenta** **Corteva Agriscience** **Bayer Crop Science** **BASF** **U. South Florida**	Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP), developing a risk-based weight of evidence (WOE) analysis framework specifically for use in evaluating the need for chronic/carcinogenicity testing in rodents for pesticide active ingredients	Partners

Input Received

NTP has provided chemical-specific hazard information for predicting human carcinogens and protecting public health for over 40 years and remains committed to providing trusted scientific information to inform public health decision-making. DNTP is committed to a transparent and collaborative process as we begin to explore new approaches for characterizing cancer hazard and risk. Opportunities for input into this process have and will continue to be provided at the NTP Board of Scientific Counselors meetings, topic-specific workshops, meetings of relevant professional organizations, and through the DNTP website. Input received to date through these venues has largely been supportive of the overall goal to develop more human-relevant and timely approaches for cancer hazard assessment and particularly for work on cancer-driver genes and mutational signatures. Common concerns voiced by stakeholders are that they will lose a trusted source of information they have become comfortable with and that new approaches will not provide information as reliable or relevant as existing methods. Consequently, questions regarding processes for establishing confidence in these new approaches, including the choice of reference data (rodent or human), are often raised but cannot be universally addressed as each project will have a different purpose and context of use and therefore require a different approach to validation. We believe that transparency and stakeholder engagement in the processes used for establishing confidence will help mitigate these concerns.

Milestones and Metrics

The Carci HEI program will continue to evolve as input is received from stakeholders and new knowledge of human cancer and carcinogens is incorporated. Accordingly, milestones and metrics are primarily focused on short-term projects aligned to longer-term goals. All work is not represented here, but examples include:

1. Develop and validate fit-for-purpose in vitro systems.
    - A sustainable culture system using metabolically competent cells has been developed using 3D HepaRG Spheroids.[3]

[3] Ramaiahgari *et al*. (2017) Three-Dimensional (3D) HepaRG Spheroid Model with Physiologically Relevant Xenobiotic Metabolism Competence and Hepatocyte Functionality for Liver Toxicity Screening. Toxicological Sciences 159: 124–136. https://doi.org/10.1093/toxsci/kfx122

- Collaboration with NC3Rs / CRACK IT Challenge is underway to develop and validate complex in vitro system for cancer hazard assessment started in 3Q2020, with an expert group meeting planned for 2Q2021, and project selection and funding in 1Q2022.
- Evaluation of temporal chemical-induced changes in cfDNA and biomolecular cargo (i.e., DNA, RNA, miRNA, protein) contained in exosomes will start in 1Q2021, looking at mutation changes over time that correspond to transformation of healthy cells into cancer cells; post-doctoral position has been funded to support this work.

2. Evaluate in vitro systems and associated data aligned to the KCCs.
   - Assays from the Tox21 high-throughput screening (HTS) program have been mapped to the KCCs and are available in the NICEATM ICE database,[4] enabling the evaluation of KCC activity for ~10,000 chemicals.
3. Develop, evaluate, and facilitate the adoption of assays and approaches that use knowledge of human cancer genetics to assess the ability of chemicals to induce tissue-specific cancers in humans.
   - PIRC rat and colonoid projects will begin 2Q2021 and be completed in 2023.
   - TwinStrand Biosciences and Wellcome Sanger collaboration will start in 2Q2021 and be completed in 2023.
   - Wellcome Sanger and Ramazzini collaboration will begin in 2Q2021 and be completed in 2024.
   - Mutational signature profile of known and suspected human carcinogens in mice has been published.[5]
   - Publications to be submitted in 2021: Comprehensive molecular characterization of mitochondrial genomes in spontaneous and chemical-induced hepatocellular carcinomas in B6C3F1/N mice; Genetic profiling of rat gliomas due to cell phone radiofrequency radiation exposure using a targeted next-generation sequencing panel (rGlioSeq); Whole-exome analysis reveals unique mutational spectra and driver genes in spontaneous and chemical-induced hepatocellular carcinomas in B6C3F1/N mice; Integrated transcriptomic analysis and identification of unique biomarkers to differentiate HCC etiologies in B6C3F1/N mouse; and Evaluation of alternative splicing and RNA signatures of spontaneous and chemical-induced hepatocellular carcinomas in B6C3F1/N mice.
4. Develop a dedicated platform for querying and exporting data from NTP cancer studies and other relevant datasets.

   The NICEATM ICE database currently contains:

   - Chemical reference lists with associated calls/classifications for all chemicals evaluated by IARC and the NTP Report on Carcinogens is currently available. An updated list with EPA's Office of Pesticide Programs classifications will be available 1Q2021.
   - A list of chemicals run in DNTP bioassays, with the level of evidence call available for each (both species, both sexes), InChiKey, QSAR Ready SMILES, and indication of Tox21 HTS data availability (n = 472 chemicals with both Tox21 HTS and rodent bioassay data). This list will be updated

[4] ICE: Integrated Chemical Environment. National Toxicology Program. https://ntp.niehs.nih.gov/go/niceatm-ice
[5] Riva *et al*. (2020) The mutational signature profile of known and suspected human carcinogens in mice. Nature Genetics 52: 1189-1197. https://doi.org/10.1038/s41588-020-0692-4

1Q2021 to include genotoxicity data, highest dose tested, dose and tissue used for level of evidence call, type of lesion, and associated p value.

- A reference list of 60 genotoxic chemicals is currently available and will be significantly increased 2Q2021 to include all chemicals tested at DNTP and additional curated data from the literature and other organizations (e.g., European Commission's Joint Research Centre (JRC) The European Union Reference Laboratory for Alternatives to Animal Testing (EURL- ECVAM), OECD).
- Curated carcinogenicity data sets are currently being incorporated into OrbiTox, a 3D visualization and analysis platform for assimilating chemical and biological data (including curated carcinogenicity data sets) from a large array of diverse data sets (chemical, genetic, pathway, and in vivo data) paired with tens of millions of experimental data points from in silico models.

Value Proposition and Summary

DNTP is the primary organization in the United States performing independent and high-quality cancer hazard characterizations for environmental chemicals. DNTP has strong expertise in genetic toxicology, pathology, and molecular approaches that can enable advances in assessing cancer risks and mechanisms. DNTP projects are often complex projects and are conducted to fulfill the needs of stakeholders, such as regulatory agencies. In addition, existing partnerships and collaborations with the Tox21 Consortium, ICCVAM agencies, OECD, IARC, and HESI, among others, provide an opportunity to leverage collaborative development of computational, alternative, and high-throughput testing strategies. Traditional cancer studies at DNTP are rigorously peer reviewed and ensure high-quality data, but it can take a long time to release actionable information necessary for risk assessment and the relevance to human health is not always certain. Therefore, introducing efficient and human-relevant methodologies earlier in the cancer testing program helps to ensure that key findings on environmental cancer hazards are available in a timelier manner, providing the opportunity for interventions that could help reduce the enormous impact these diseases have on public health and the economy.