Carcinogenicity Health Effects
Innovation Program

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Division of the NTP
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
February 2, 2021
**Why do we focus on cancer?**

**Cancer is widespread**

Cancer, all invasive sites, US, 2014-2016


Leading causes of death, US, 2018

<table>
<thead>
<tr>
<th>Cause</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease</td>
<td>655,341</td>
</tr>
<tr>
<td>Cancer</td>
<td>599,265</td>
</tr>
<tr>
<td>Chronic lower respiratory</td>
<td>159,481</td>
</tr>
<tr>
<td>Accidents (unintentional injury)</td>
<td>167,107</td>
</tr>
<tr>
<td>Stroke (cerebrovascular)</td>
<td>147,809</td>
</tr>
<tr>
<td>Alzheimer's</td>
<td>122,018</td>
</tr>
<tr>
<td>Diabetes</td>
<td>84,940</td>
</tr>
<tr>
<td>Pneumonia &amp; influenza</td>
<td>59,118</td>
</tr>
<tr>
<td>Nephritis &amp; Nephrosis</td>
<td>51,383</td>
</tr>
<tr>
<td>Intentional self-harm (suicide)</td>
<td>48,344</td>
</tr>
</tbody>
</table>


Rates of new cases and death from cancer are both decreasing

Incidence rates dropped 14% from 1998-2017 (20 years)

Death rates dropped 26% from 1999-2018 (20 years)

Seer.Cancer.gov
There is still plenty of room for improvement

Environmental risk factors are critical in cancer prevention → Carci HEI focuses on environmental exposure contribution to cancer

Intrinsic risk factors contribute only modestly (less than ~10–30% of lifetime risk) to cancer development

Cancers are preventable
UK, 2015

Nature 529, 43–47(2016)
https://www.nature.com/articles/nature16166/

https://www.cancerresearchuk.org/health-professional/cancer-statistics/risk/preventable-cancers
Health disparity is being investigated (under NIEHS-wide Environmental Health Disparity Faculty)

There is still plenty of room for improvement

African Americans in the US have the highest death rate of any racial or ethnic group

Cancer incidence and death rates by race/ethnicity, US, 2014-2018

- **American Indian/Alaska Native**: 141.1
- **Asian/Pacific Islander**: 97.2
- **Hispanic**: 110.8
- **Black**: 177.5
- **White**: 156.3
- **All Races**: 155.5

U.S. Mortality Rate
SEER 21 Rate of New Cases

Rate per 100,000
There is still plenty of room for improvement

Some cancers have increasing rates of new cases and death →
A need for researching site-specific cancers

**National Trends in rates of new cancer cases**
**US, 2012-2016**

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Average annual percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver &amp; Intrahepatic Bile Duct</td>
<td>-2.6%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>-0.5%</td>
</tr>
<tr>
<td>Kidney &amp; Renal Pelvis</td>
<td>0.1%</td>
</tr>
<tr>
<td>Oral Cavity &amp; Pharynx</td>
<td>-0.2%</td>
</tr>
<tr>
<td>Prostate</td>
<td>-0.4%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>-0.6%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>-1.1%</td>
</tr>
<tr>
<td>Stomach</td>
<td>-1.4%</td>
</tr>
<tr>
<td>Bladder</td>
<td>-1.6%</td>
</tr>
<tr>
<td>Lung &amp; Bronchus</td>
<td>-2.1%</td>
</tr>
<tr>
<td>-5</td>
<td>-4</td>
</tr>
</tbody>
</table>

**National Trends in cancer death rates**
**US, 2012-2017**

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Average annual percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver &amp; Intrahepatic Bile Duct</td>
<td>-6.3%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>-6.1%</td>
</tr>
<tr>
<td>Kidney &amp; Renal Pelvis</td>
<td>-1.3%</td>
</tr>
<tr>
<td>Oral Cavity &amp; Pharynx</td>
<td>-1.1%</td>
</tr>
<tr>
<td>Stomach</td>
<td>-2.9%</td>
</tr>
<tr>
<td>Bladder</td>
<td>-2.1%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>-2.3%</td>
</tr>
<tr>
<td>Lung &amp; Bronchus</td>
<td>-2.6%</td>
</tr>
<tr>
<td>Ovary</td>
<td>-2.7%</td>
</tr>
<tr>
<td>-5</td>
<td>-4</td>
</tr>
</tbody>
</table>

* Significantly different from zero (p<0.05)

Seer.Cancer.gov
New approach

Image credit: (YouTube) Kurzgesagt - In a Nutshell.

NTP has expertise and the will to take on this challenge

**Have expertise in**
- Traditional rodent cancer studies
- Genetic toxicology, pathology, and molecular approaches
- Existing partnerships and collaborations

**Well positioned to**
- Make data accessible and integrated with other types of data
- Enable advances in assessing cancer risks and mechanisms
- Collaboratively develop alternative and high-throughput testing/evaluation strategies

**Accomplish**
- Innovation in testing & assessment
  - Efficient and human-relevant methodologies earlier in the cancer testing program
- Key findings on environmental cancer hazards are available in an efficient manner
  - Opportunity for interventions
Objective 1 - New approaches for cancer hazard assessment

Objective 2 - Investigation of tissue specific human cancers

Objective 3 - Resources to make existing information on carcinogens Findable, Accessible, Interoperable and Reusable (FAIR)

Objective 4 - Collaborations and stakeholder engagement
Examining chemical carcinogenesis – Current paradigm

Throughput

Human relevance

Chemical initiator

Molecular Toxicology

Adverse Outcome Pathway

Increasing levels of biological organization

Molecular Pathology

QSAR Relationships

Cell culture and Genetox assays

Organoids, metabolically competent

Lower order model organisms

Rodent models

https://www.epa.gov/sites/production/files/2016-10/aop1.png
Objective 1: New approaches for cancer studies

Translation
Conserved Biology
• Human relevance

Mechanisms
Integrated -omics approaches
• Molecular pathways
Identify biomarkers
• Exposure
• Neoplasia

Prediction
Short-term in vivo screens
• Epigenetic landmarks
• Mutation signatures
• Gene expression

in vitro screens
• Immortalized cells
• 3D cell culture

Adapted from The Cancer Genome Atlas Research Network, 2017
Objective 1: New approaches for cancer studies

Evaluating genomic alterations leading to neoplasia

• Whole genome sequencing of 188 spontaneous or chemical induced mouse tumors
  – NTP’s collaboration with Allan Balmain (UCSF) and David Adams (Sanger Institute) as part of the Cancer Research UK (CRUK) Grand Challenge grant

• Multi-omics evaluation of 140 mouse hepatocellular carcinomas (HCCs)
  – Whole exome sequencing, high depth RNA sequencing and miRNA sequencing
Mutation signatures are conserved between rodent and human tumors.

Only ~15% of chemical-induced tumors had mutation signatures distinct from those of spontaneous tumors.

Endogenous mutational processes (and tumor promotion pathways) are diverse.

Identified cancer driver genes in rodent tumors:
- Kras, Hras, Fgfr2, Ctnnb1, Braf, Egfr, Sfr1, and Ube2c.

Identified unique isoforms in cancer genes in mouse HCCs:
- ↑ Ncor2, Nrg1, Tgfbr2; ↓ Ptprd.

Identified IncRNAs and miRNAs uniquely altered in mouse HCCs.

Riva, Pandiri, Li, et al., 2020 Nature Genetics; Xu et al.; Manuscript in preparation.
Partnerships and novel approaches to examine carcinogenesis

• Expand multi-omics investigations on rodent tumors exposed to diverse chemical classes and continue collaborations with Sanger institute
  – Explore rodent tissue archives from Ramazzini institute (Italy), academic and industry partners

• Novel approaches with a focus on mechanisms, translation and prediction
  – Human relevant in vitro approaches including metabolism competent organoids
  – Design subchronic studies to gain more mechanistic information (along with traditional endpoints) to address data gaps in adverse outcome pathways (AOPs)
  – Cancer driver gene mutation panels using error corrected duplex sequencing technology
  – Screening panels to detect cancer-specific splice variants, IncRNA and miRNA
  – Collaborate with stakeholders to evaluate in vitro assays aligned to Key Characteristics of Carcinogens (KCCs)
  – Contribute to HESI initiatives: Carcinogenomics and Genetic toxicology
Objective 1 - New approaches for cancer hazard assessment

**Objective 2 - Investigation of tissue specific human cancers**

Objective 3 - Resources to make existing information on carcinogens Findable, Accessible, Interoperable and Reusable (FAIR)

Objective 4 - Collaborations and stakeholder engagement
Objective 2 - Investigation of tissue specific human cancers

Early onset colorectal cancers (EO-CRC)

• Increasing incidence rates of colorectal cancer in younger demographics

• NCI-NIEHS sponsored a thinktank on EO-CRC
  – Environmental exposures are a primary concern

• Carci HEI developed projects that align with the NTP translational toxicology pipeline

Early-onset colorectal cancer research: gaps and opportunities

Laura Brockway-Lunardi¹, Stefanie Nelson², Arun R Pandiri³, James V Tricoli⁴, Asad Umar⁵, Anil Wali⁶ & Phillip J Daschner*⁷

Siegel et al., 2020
Hypotheses Driven Research

- Literature review
- Mutation signature analysis of EO-CRC sequencing data
- Computational Toxicology
- Profiling based on suspected human CRC carcinogens
- Development of colonoids from the PIRC* rat and human cells
- Screening of potential compounds identified by in silico approaches
- Screening of potential compounds identified by in silico approaches
- In vitro Screening
- Knowledge integration
- Chronic in vivo Studies
- Short-term in vivo Studies
- Communication

Comparison of mutation signatures and molecular pathways from in vitro and short-term animal studies with the human data

Human Health Effects

- CPSC, FDA, EPA, NIEHS, NCI
- IARC, ORoC
- Colon Cancer Foundation
- American Cancer Society

*PIRC: Polyposis in rat colon
Leveraging TTP to bridge the translational gaps across models

- Determine mutation signatures
  - Human EO-CRCs and LO-CRCs from dbGaP and TCGA database
  - In vitro or in vivo models (such as PIRC rats)
- Develop colonoids derived from human cells (iPSC and ESC) and the PIRC rat
  - Screen potential etiologic factors implicated in CRC in humans
- In vivo studies using PIRC rats to confirm the potential to contribute to CRC
Objective 1 - New approaches for cancer hazard assessment

Objective 2 - Investigation of tissue specific human cancers

Objective 3 - Resources to make existing information on carcinogens Findable, Accessible, Interoperable and Reusable (FAIR)

Objective 4 - Collaborations and stakeholder engagement
Objective 3 - Resources to make existing information on carcinogens FAIR

- **Curated data and search tools**
  - Organized by toxicity endpoints
  - Standardized terminology, units, and formatting

- **Curated chemical lists**
  - Reference lists with classifications and bioactivity
  - In vitro assays linked with defined terminology

- **Computational models**
  - In vitro to in vivo extrapolation (IVIVE)
  - Quantitative structure-activity relationship (QSAR) models

Chemical Effects in Biological Systems (CEBS)
https://manticore.niehs.nih.gov/cebssearch

Integrated Chemical Environment (ICE)
https://ice.ntp.niehs.nih.gov/
Integrated Chemical Environment (ICE) database

Chemical Lists

Tox21 HTS assays mapped to Key Characteristics of Carcinogens (KCC)

- genotoxicity data
- highest dose tested
- dose and tissue used for level of evidence call
- type of lesion

Additional information on each chemical to be added 1Q2021
Objective 1 - New approaches for cancer hazard assessment

Objective 2 - Investigation of tissue specific human cancers

Objective 3 - Resources to make existing information on carcinogens Findable, Accessible, Interoperable and Reusable (FAIR)

Objective 4 - Collaborations and stakeholder engagement
Objective 4 - Collaborations and stakeholder engagement

• Need for new communication strategy
• New approaches to carcinogenicity testing and assessment
  – How different are they from the 2-year bioassay
  – Value and limitations of the new approaches
  – Overcoming challenges to adapt the new approaches
    • Better communication and engagement during development: white papers, workshops
• Optimally use all communication channels
  – Social media, peer reviewed journals, updates on the NTP webpage
  – Expand target audience by including traditional stakeholders and lay public
Stakeholders

Institution Type
- Federal Agency

Stakeholder Type
- Partner
- Collaborator
- Contributor
- User
# Timeline of selected projects

<table>
<thead>
<tr>
<th>Development and validation of fit-for-purpose in vitro systems</th>
<th>2020</th>
<th>2021</th>
<th>2022</th>
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<tbody>
<tr>
<td>3D HepaRG spheroid</td>
<td>Q1</td>
<td>Q2</td>
<td>Q3</td>
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<tr>
<td>Develop &amp; validate complex in vitro system for cancer hazard</td>
<td>Q3</td>
<td>Q4</td>
<td></td>
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<td>Changes in exosome, mutation</td>
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<tr>
<td>专家会议</td>
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<td>Q1</td>
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<tr>
<td>项目选择和资金支持</td>
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<td>Q2</td>
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<table>
<thead>
<tr>
<th>Evaluation of in vitro systems &amp; data</th>
<th>2020</th>
<th>2021</th>
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<tbody>
<tr>
<td>Tox21 assays mapped to KCCs and presented in ICE</td>
<td>Q1</td>
<td>Q2</td>
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</table>

<table>
<thead>
<tr>
<th>Assays and approaches that use</th>
<th>2020</th>
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</thead>
<tbody>
<tr>
<td>Mutation signature in mice</td>
<td>Q3</td>
</tr>
<tr>
<td>PIRC rat and colonoid projects</td>
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<tr>
<td>TwinStrand Biosciences and</td>
<td></td>
</tr>
<tr>
<td>Sanger collaboration</td>
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<tr>
<td>公布</td>
<td>Publication</td>
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</table>

<table>
<thead>
<tr>
<th>Platform for querying and exporting data from NTP</th>
<th>2020</th>
<th>2021</th>
</tr>
</thead>
<tbody>
<tr>
<td>Updated list with RoC, IARC, and EPA IRIS</td>
<td>Q1</td>
<td>Q2</td>
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<tr>
<td>classifications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Updated list with EPA OPP classifications</td>
<td>Q2</td>
<td>Q3</td>
</tr>
<tr>
<td>Updated list with genotoxicity data, highest dose</td>
<td></td>
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<tr>
<td>tested, etc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Include all chemicals tested at DNTP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and additional curated data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity data into OrbiTox</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Updated list with RoC, IARC, and EPA IRIS classifications
- Project selection and funding
Thank you!!