NTP
National Toxicology Program

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

Carci HEI Program Management Team Members

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Predictive Toxicology Branch

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Comparative and Molecular Pathogenesis Branch

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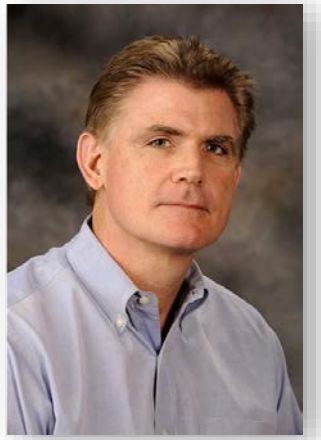
Systems Toxicology Branch

Erik Tokar
Mechanistic Toxicology Branch



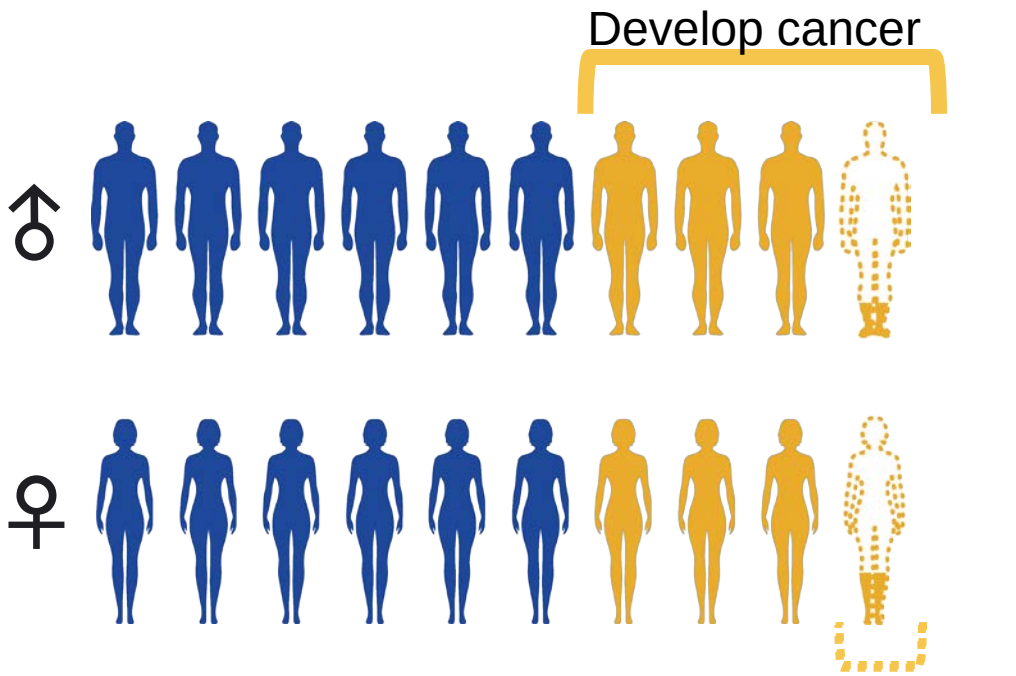
Warren Casey
Predictive Toxicology Branch
(leadership liaison)

Ian Chan
Mechanistic Toxicology Branch
(ad hoc member)

Why do we focus on cancer?

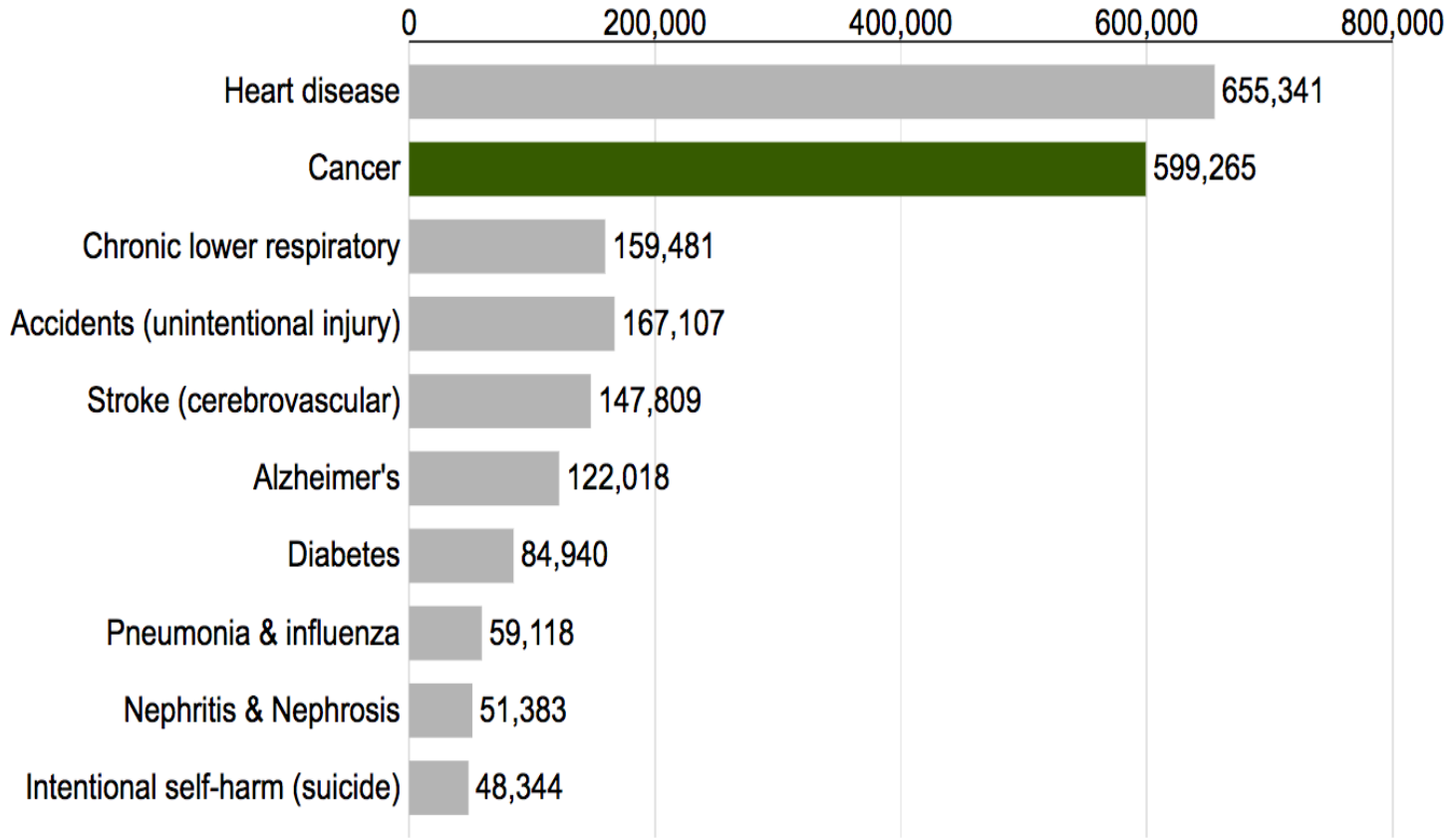
Cancer is widespread

Cancer, all invasive sites, US, 2014-2016



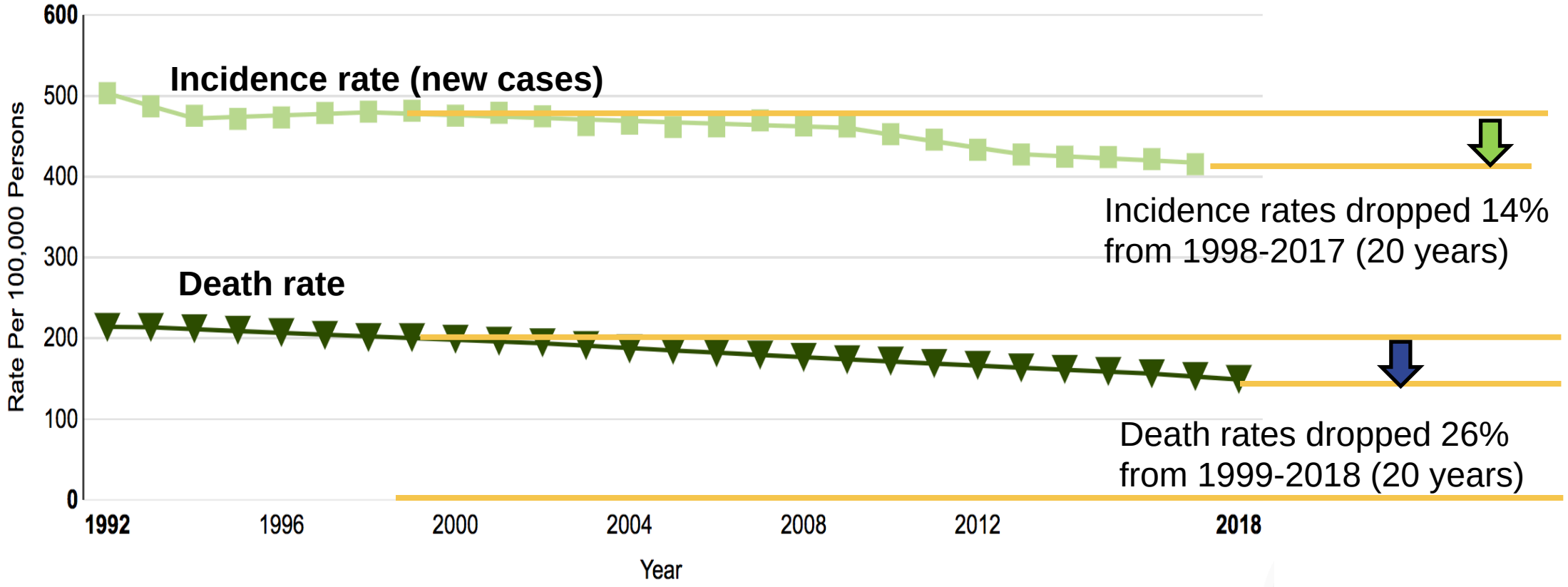
www.cancer.org
https://www.cancer.org/cancer/cancer-basics/lifetime-probability-of-developing-or-dying-from-cancer.html

Leading causes of death, US, 2018



Seer.Cancer.gov
https://seer.cancer.gov/statfacts/html/common.html

Rates of new cases and death from cancer are both decreasing



Seer.Cancer.gov
https://seer.cancer.gov/statfacts/html/all.html

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

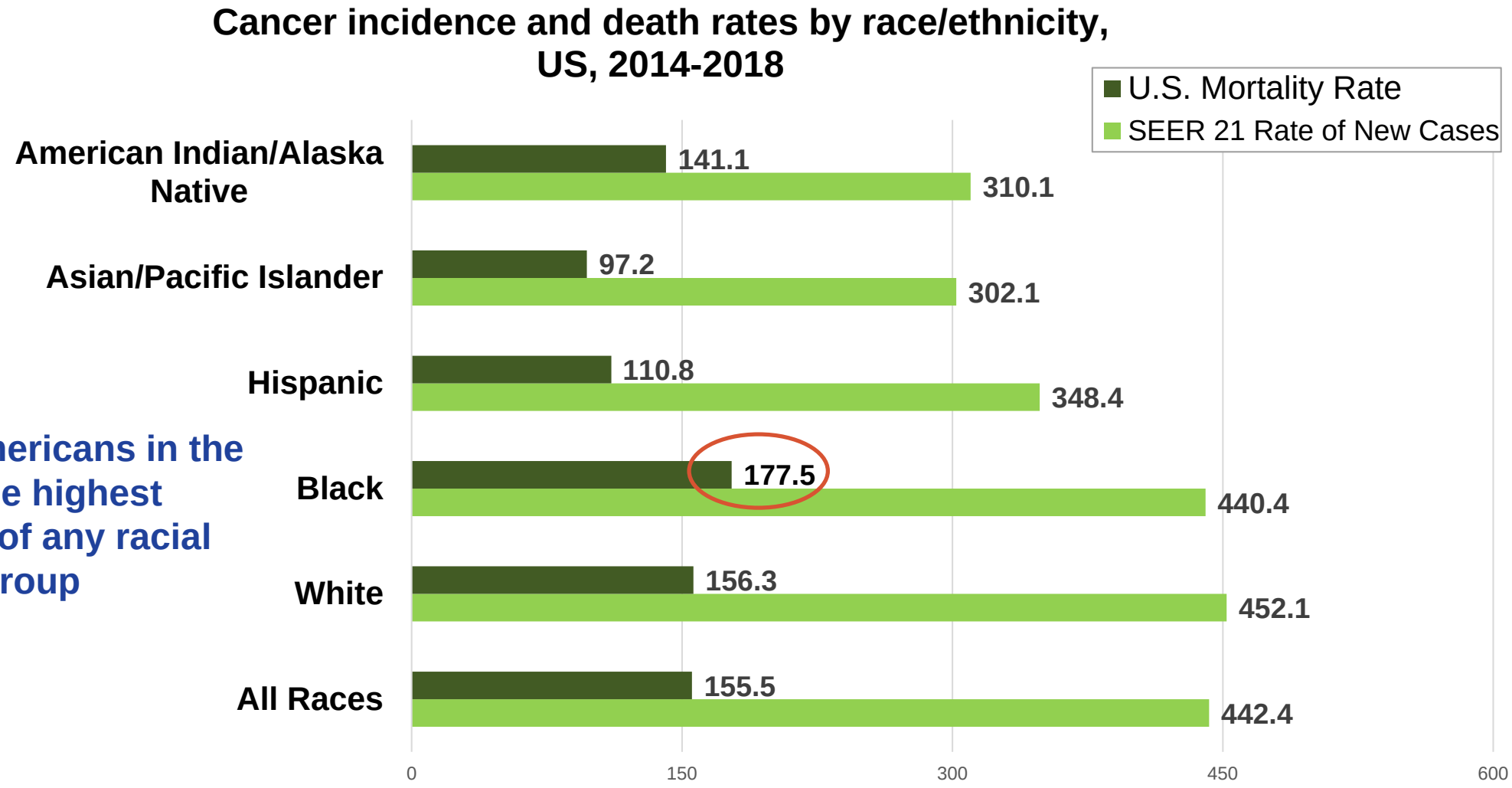
Nature 529,:43–47(2016)
https://www.nature.com/articles/nature16166/

Cancers are preventable
UK, 2015

https://www.cancerresearchuk.org/health-professional/cancer-statistics/risk/preventable-cancers

There is still plenty of room for improvement

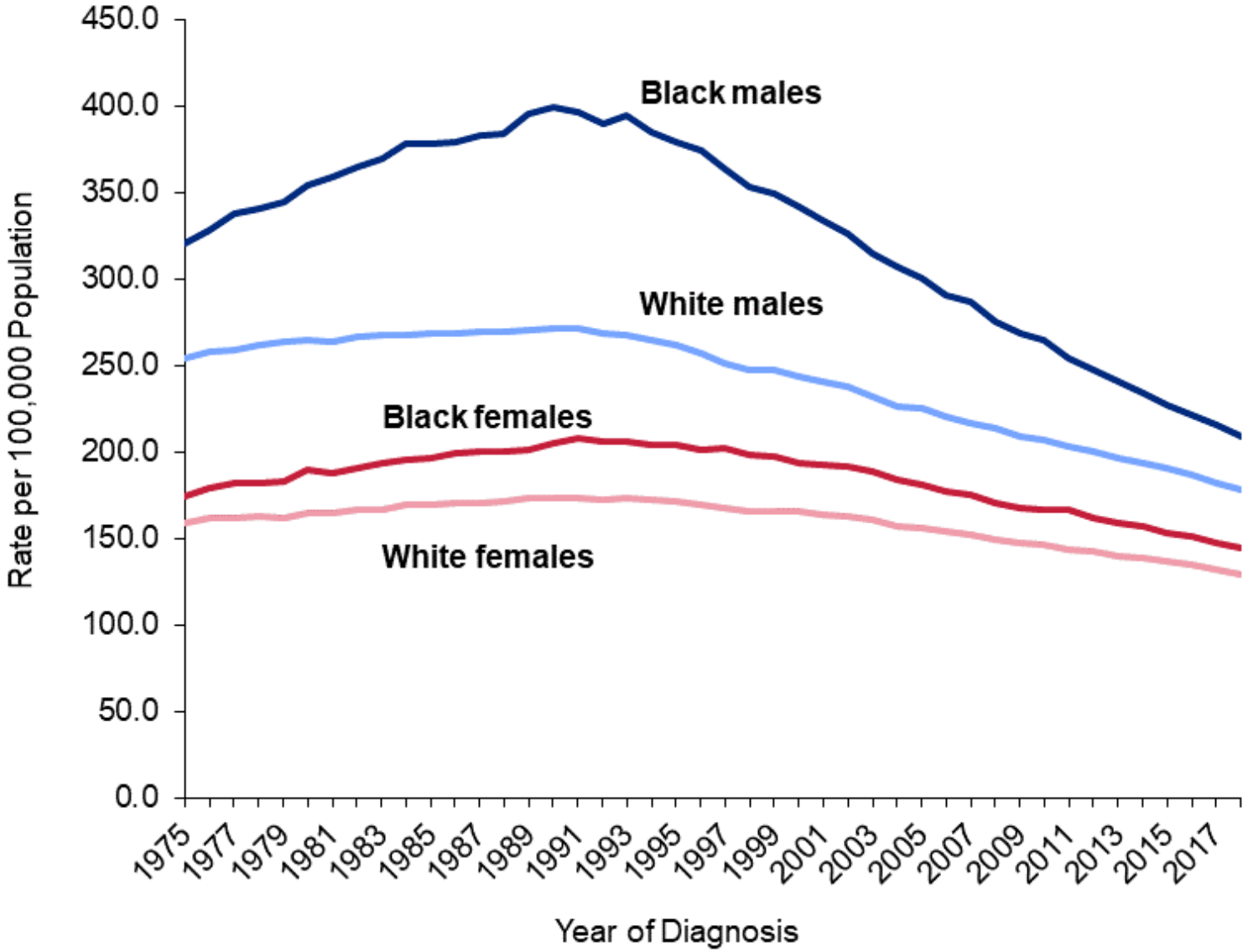
Health disparity is being investigated (under NIEHS-wide Environmental Health Disparity Faculty)



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

Seer.Cancer.gov
https://seer.cancer.gov/statfacts/html/disparities.html

Trends in cancer death rates by sex and race, US, 1975-2018

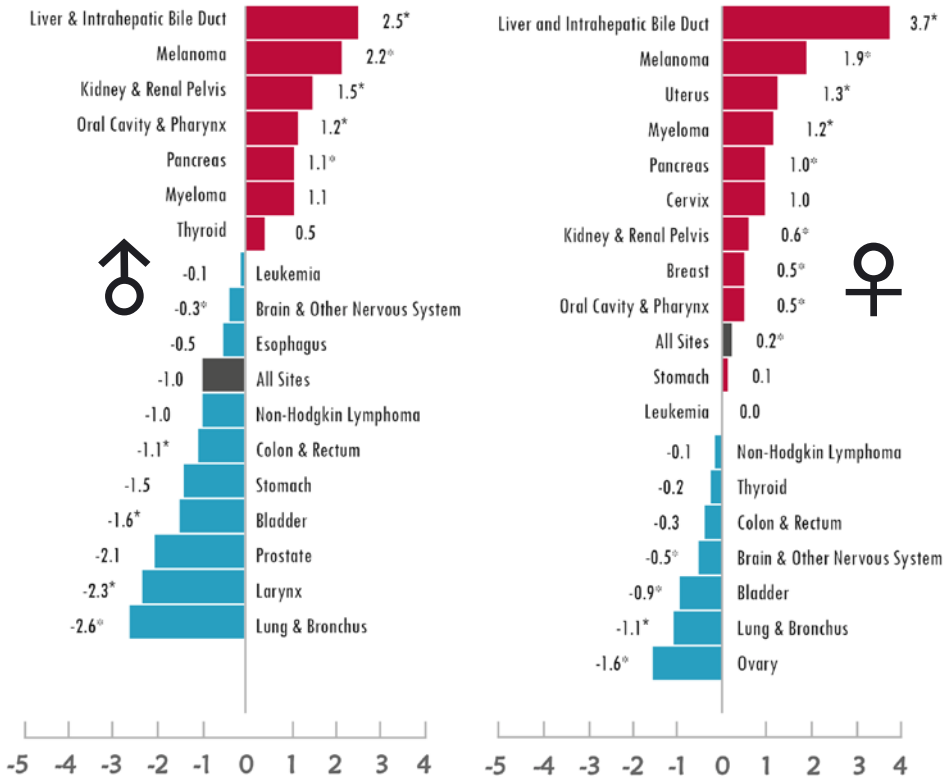


Seer.Cancer.gov
https://seer.cancer.gov/statfacts/html/disparities.html

There is still plenty of room for improvement

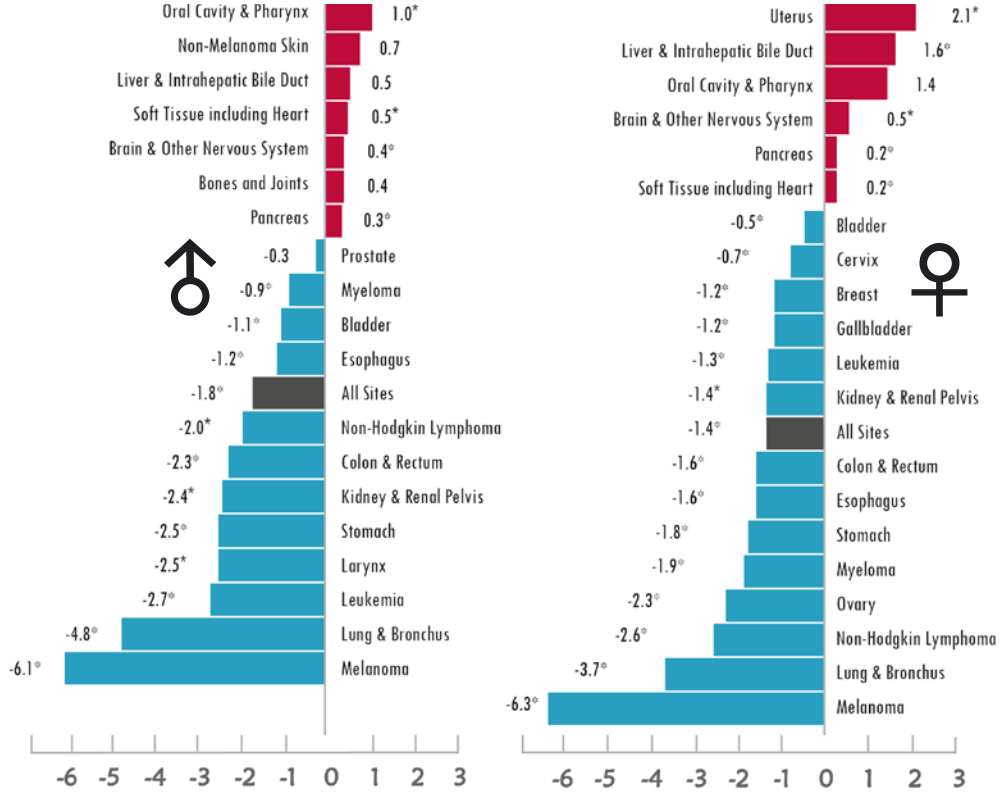
Some cancers have increasing rates of new cases and death → A need for researching site-specific cancers

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



Average annual percent change

National Trends in cancer death rates US, 2012-2017



Average annual percent change

Seer.Cancer.gov
https://seer.cancer.gov/report_to_nation/infographics/trends_mortality.html

* Significantly different from zero ($p<0.05$)

Traditional approach

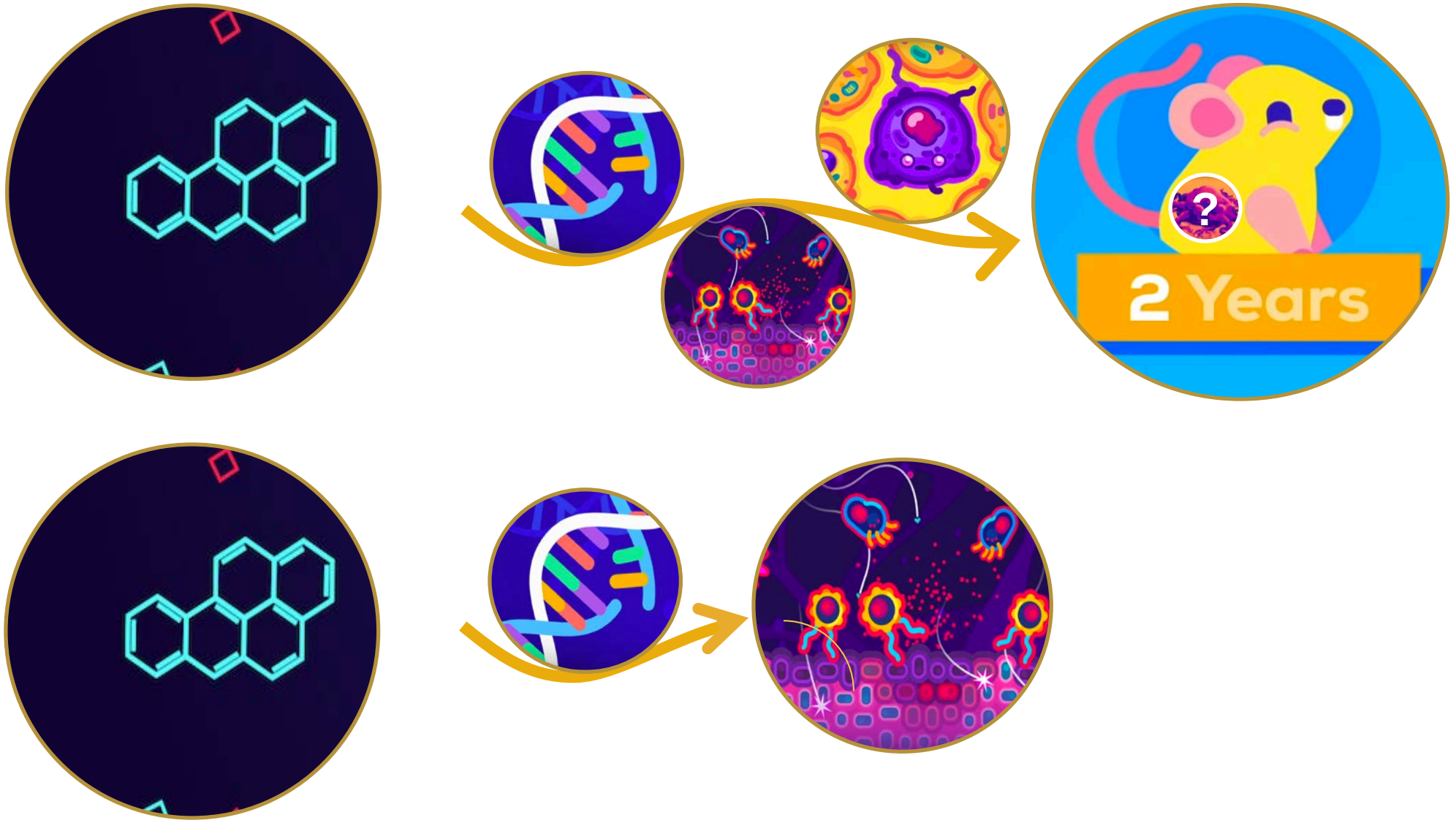


Image credit: (YouTube) Kurzgesagt – In a Nutshell

New approach

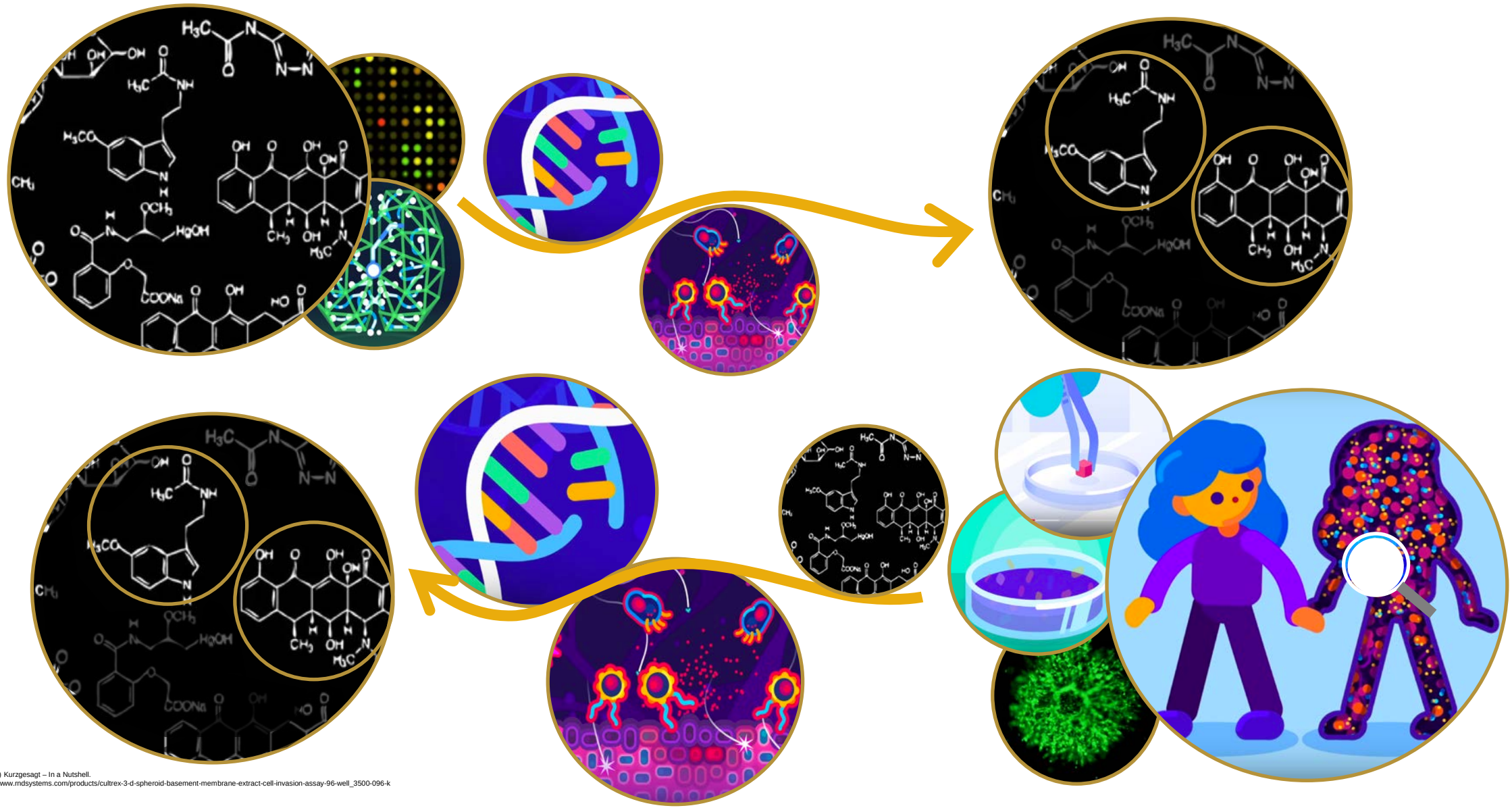


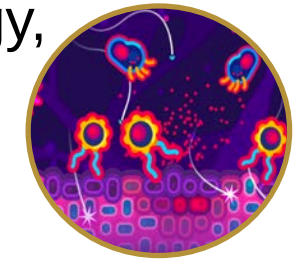
Image credit: (YouTube) Kurzgesagt – In a Nutshell.
3D cell sphere: https://www.rndsystems.com/products/cultrex-3-d-spheroid-basement-membrane-extract-cell-invasion-assay-96-well_3500-096-k

NTP has expertise and the will to take on this challenge

Have expertise in

- Traditional rodent cancer studies

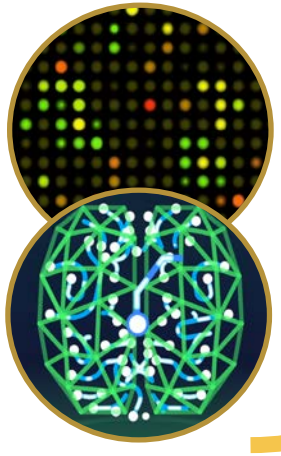
- Genetic toxicology, pathology, and molecular approaches



- 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

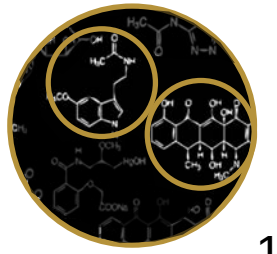


Image credit: (YouTube) Kurzgesagt – In a Nutshell

Objectives

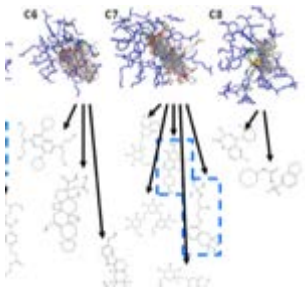
Objective 1 - New approaches for cancer hazard assessment

Objective 2 - Investigation of tissue specific human cancers

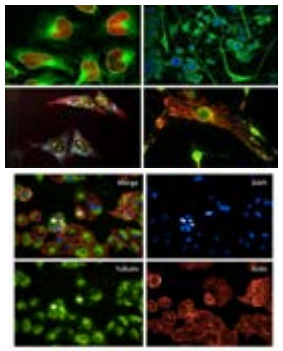
Objective 3 - Resources to make existing information on carcinogens Findable, Accessible, Interoperable and Reusable (FAIR)

Objective 4 - Collaborations and stakeholder engagement

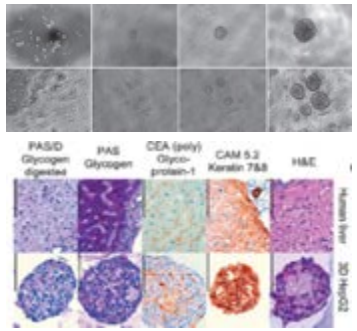
Examining chemical carcinogenesis – Current paradigm



QSAR Relationships



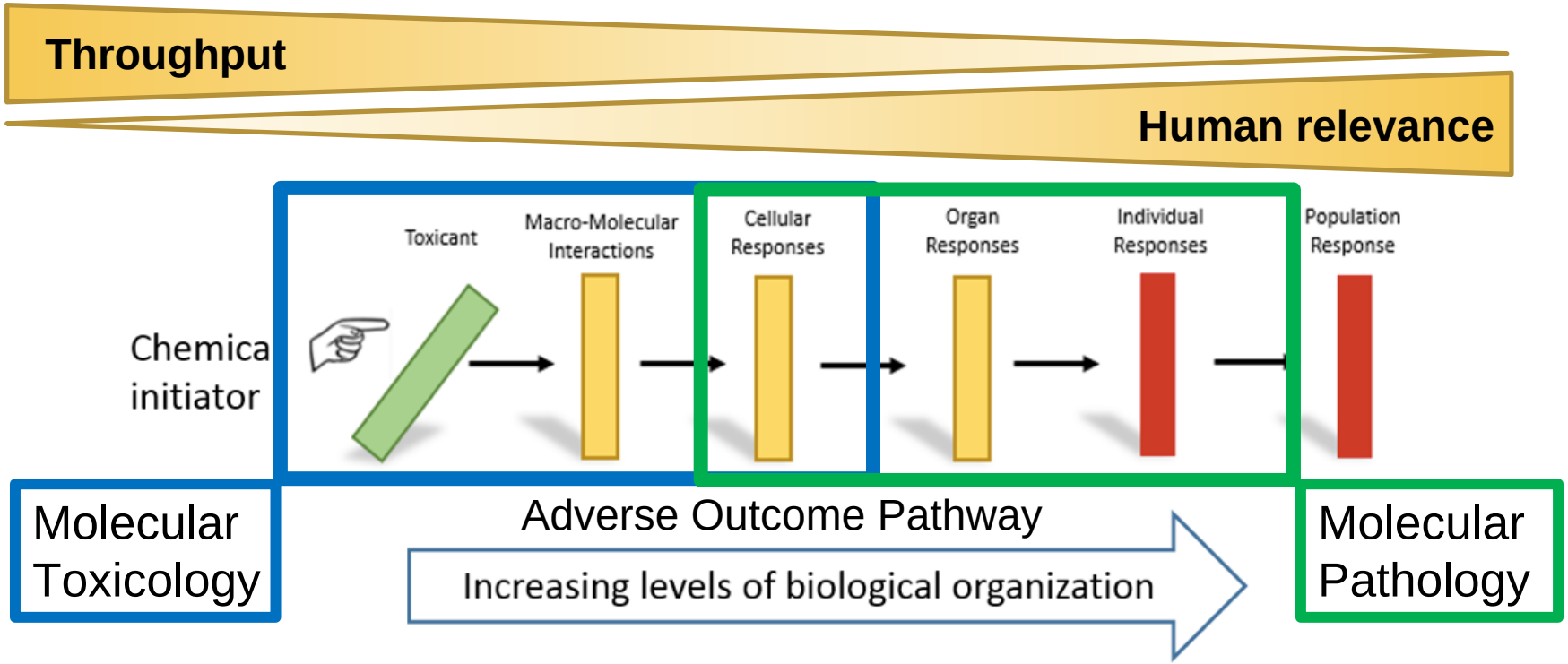
Cell culture and Genetox assays



Organoids, metabolically competent

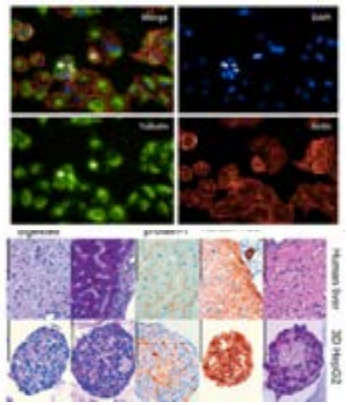
Lower order model organisms

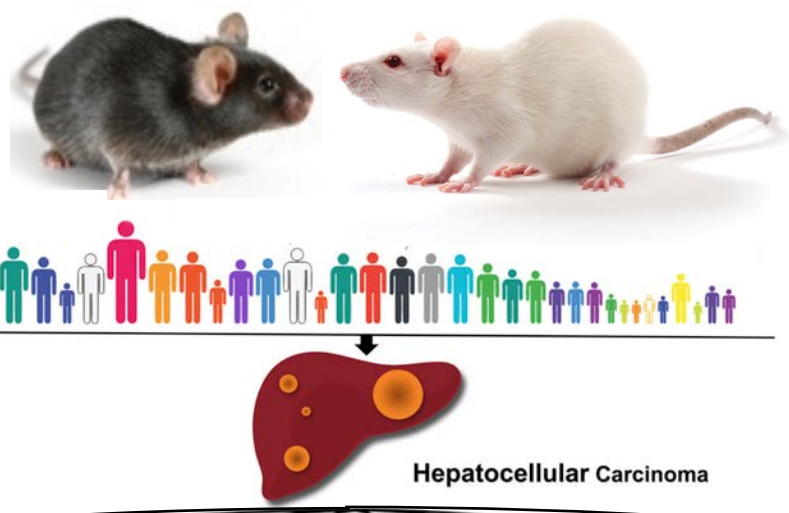
Rodent models



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

Complement Existing NTP Cancer Assessment Approaches

Adapted form 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

Summary and data update on NGS studies on mouse tumors

- 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 lncRNAs 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, lncRNA 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

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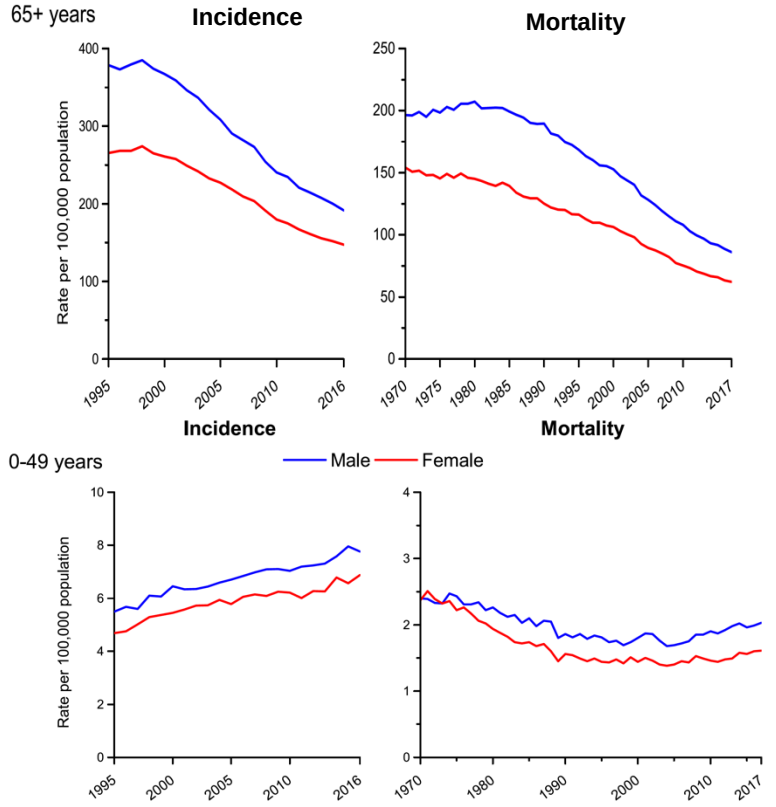
Objective 2 - Investigation of tissue specific human cancers

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



Siegel et al., 2020

Early-onset colorectal cancer research: gaps and opportunities

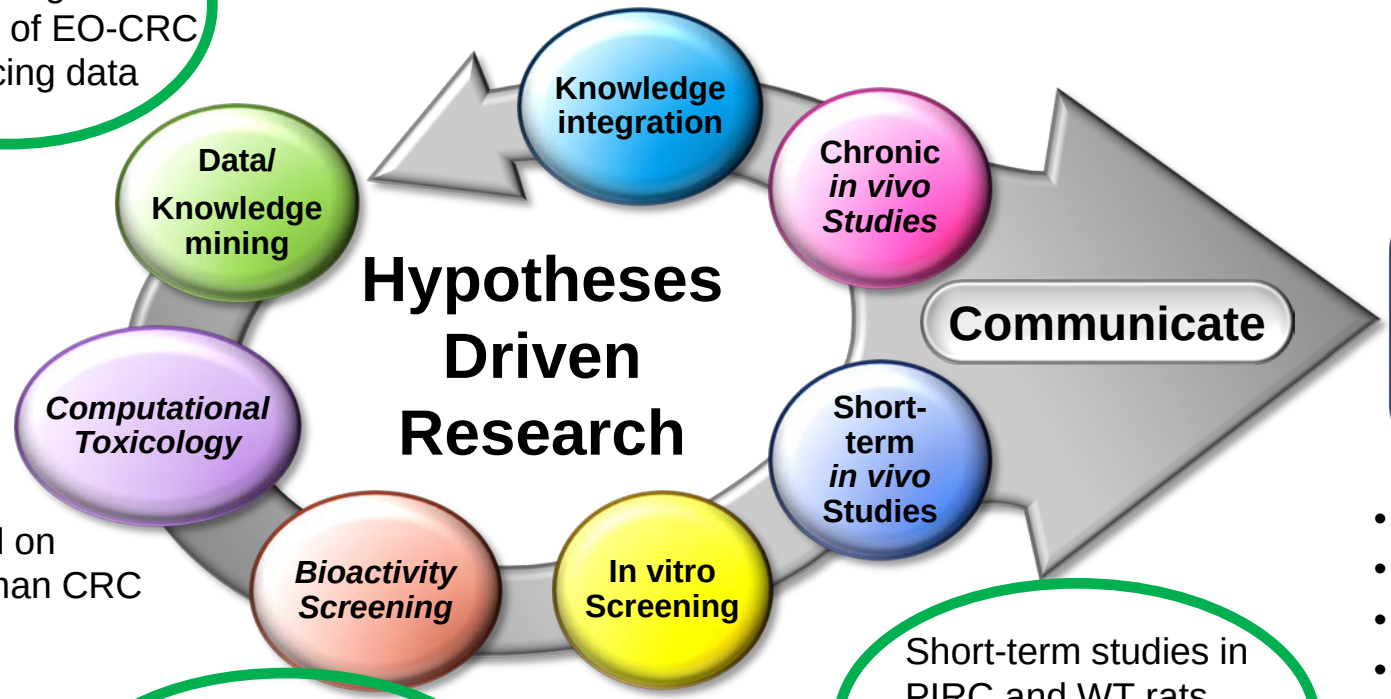
Laura Brockway-Lunardi[1], Stefanie Nelson[2], Arun R Pandiri[3], James V Tricoli[4], Asad Umar[5], Anil Wali[6] & Phillip J Daschner*,[7]

DNTP Translational Toxicology Pipeline (TTP) for EO-CRC

Literature review

Mutation signature analysis of EO-CRC sequencing data

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

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

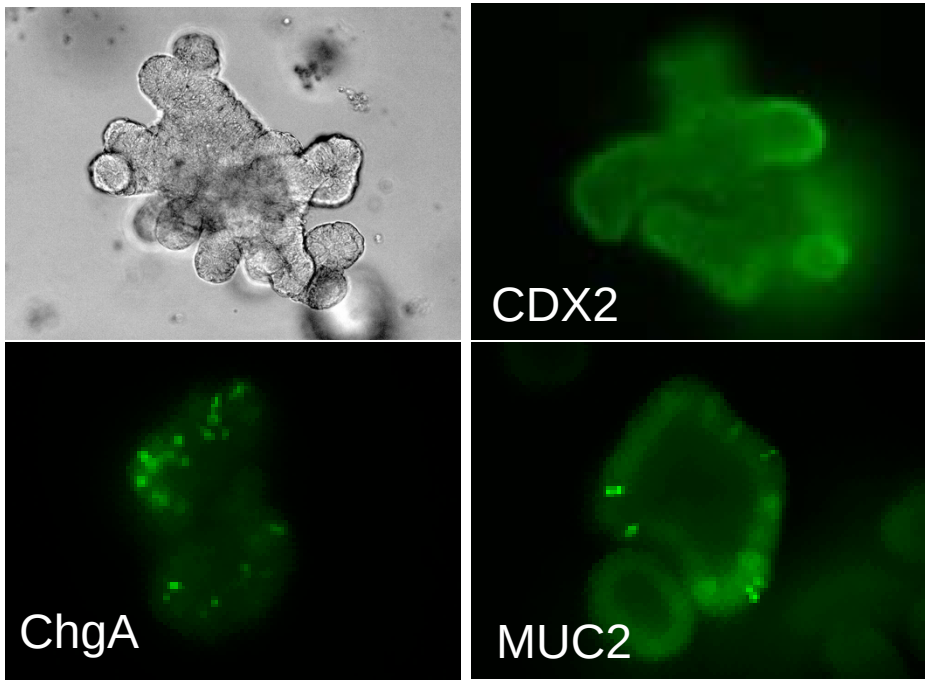
Short-term studies in PIRC and WT rats

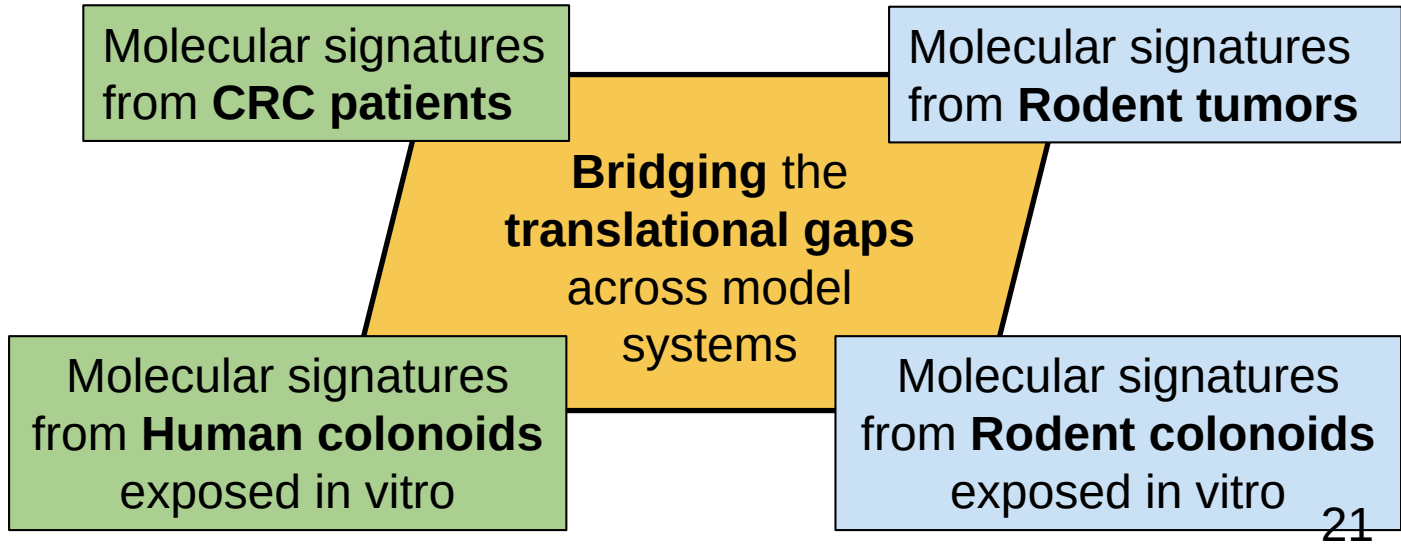
Investigate rodent CRC tissues

***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





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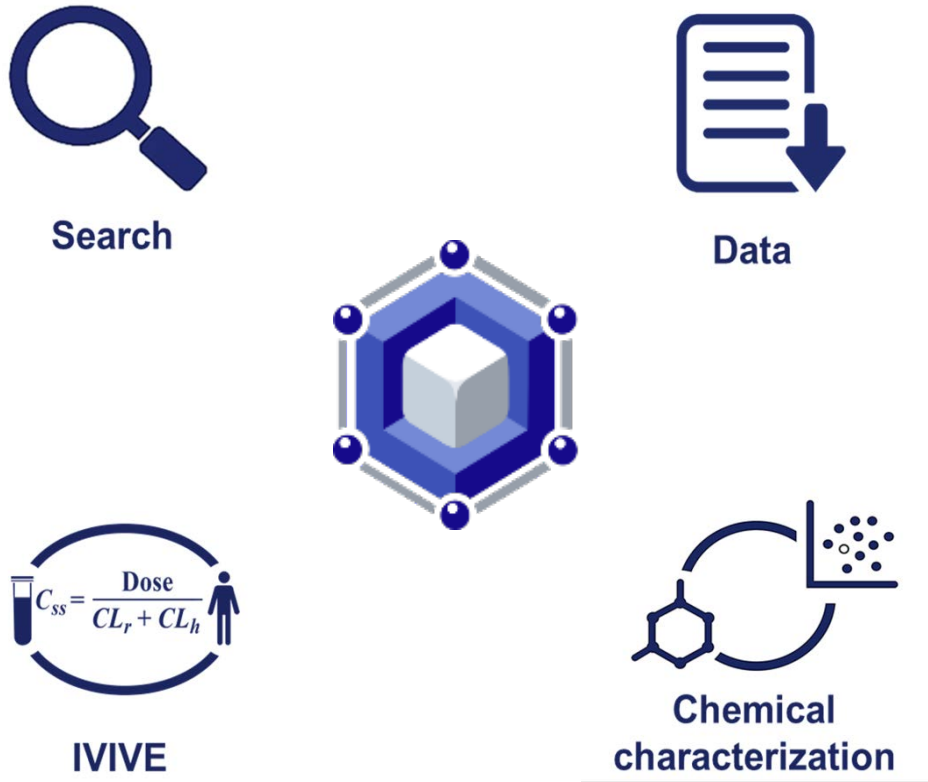
Objective 3 - Resources to make existing information on carcinogens FAIR

- 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/



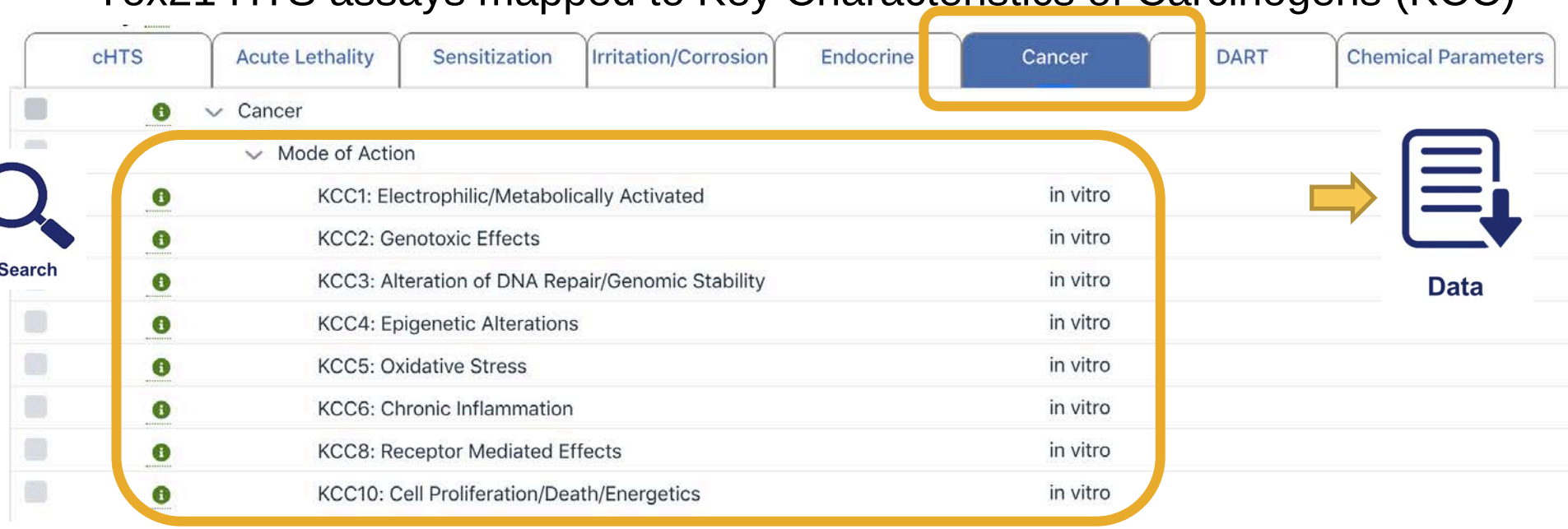
Integrated Chemical Environment (ICE) database

Chemical Lists

- Tox21
- AR In Vitro Agonist (R)
- AR In Vitro Antagonist (R)
- AR In Vivo Agonist
- AR In Vivo Antagonist
- EPA IRIS Carcinogenicity Classifications
- EPA Pesticide Active Ingredients
- EPA Pesticide Inert Ingredients, Food and Nonfood Use
- ER In Vitro Agonist (R)
- ER In Vivo Agonist (R)
- Eye Irritation-Corrosion (R)
- Genotoxicity (R)
- IARC Classifications
- NTP Cancer Bioassay Chemicals
- RoC Classifications
- Skin Corrosion (R)
- Steroidogenesis - Androgen
- Steroidogenesis - Estrogen
- Thyroid

EPA OPP to be added 1Q2021

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



CASRN	Chemical Name	DTXSID	Original SMILES	Original InChIKey	QSAR Ready SMILES	Technical Report No.	NTP Level Of Evidence Male Rats	NTP Level Of Evidence Female Rats	NTP Level Of Evidence Male Mice	NTP Level Of Evidence Female Mice	Tested in Tox21
67-66-3	Chloroform	DTXSID1020306	ClC(Cl)Cl	HEDRZPFGACZZDS-UHFFFAOYSA-N	ClC(Cl)Cl	TR-000	P	NE	P	P	Yes
143-50-0	Chlordecone (kepone)	DTXSID1020770	ClC12C(=O)C3(Cl)C4(Cl)C1(Cl)C1(Cl)C2(Cl)C3(Cl)C4(Cl)C1(Cl)Cl	LHHGDZSESBACKH-UHFFFAOYSA-N	ClC12C(=O)C3(Cl)C4(Cl)C1(Cl)C1(Cl)C2(Cl)C3(Cl)C4(Cl)C1(Cl)Cl	TR-001	P	P	P	P	Yes
79-01-6	Trichloroethylene	DTXSID0021383	ClC=C(Cl)Cl	XSTXAVWGXDQKEL-UHFFFAOYSA-N	ClC=C(Cl)Cl	TR-002	NE	NE	P	P	Yes

Additional information on each chemical to be added 1Q2021

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

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Objective 4 - Collaborations and stakeholder engagement

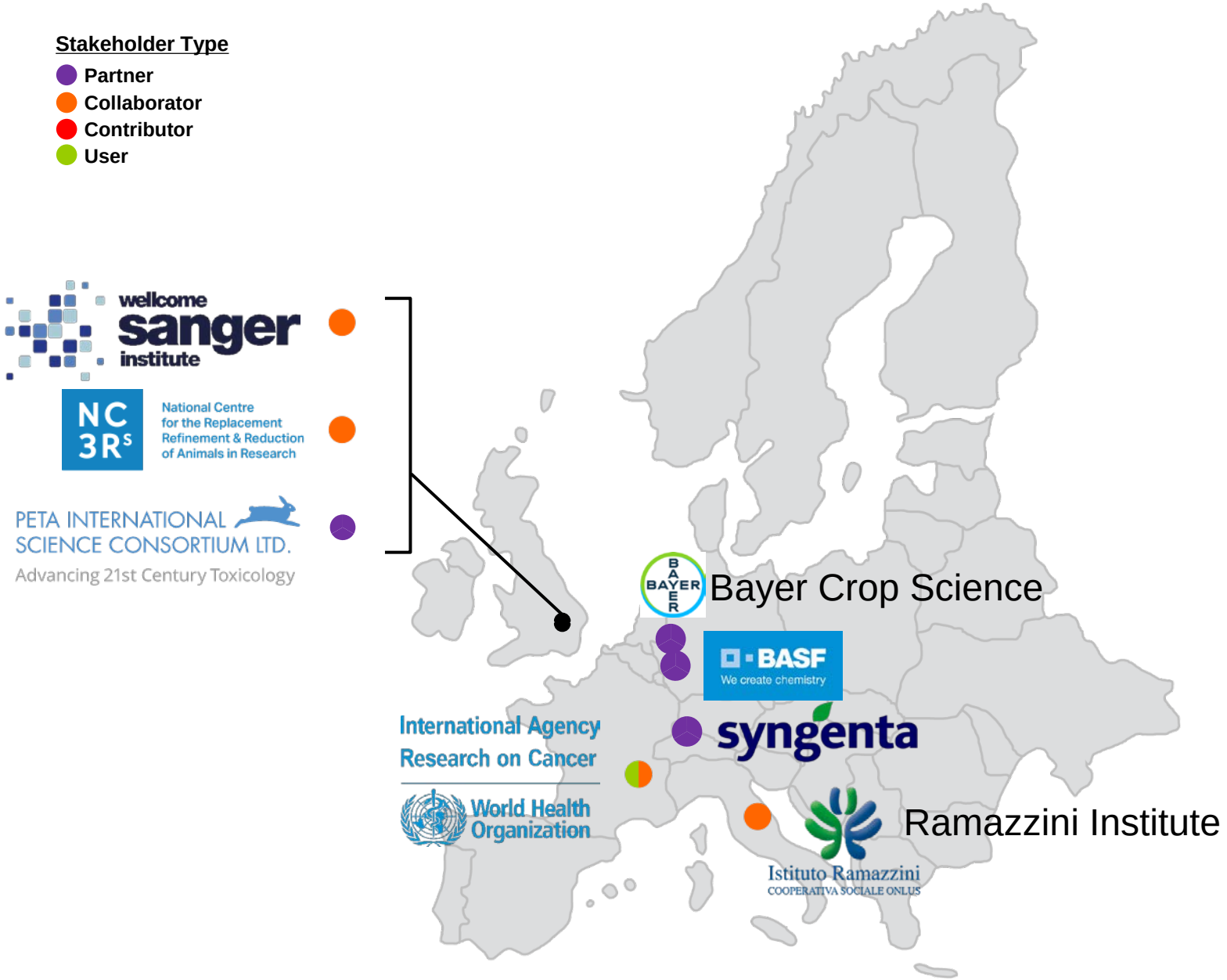
- Need for new communication strategy
- New approaches to carcinogenicity testing and assessment
  - How different are they from the 2-year bioassay
  - Value and limitations of the new approaches
  - Overcoming challenges to adapt the new approaches
    - Better communication and engagement during development: 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

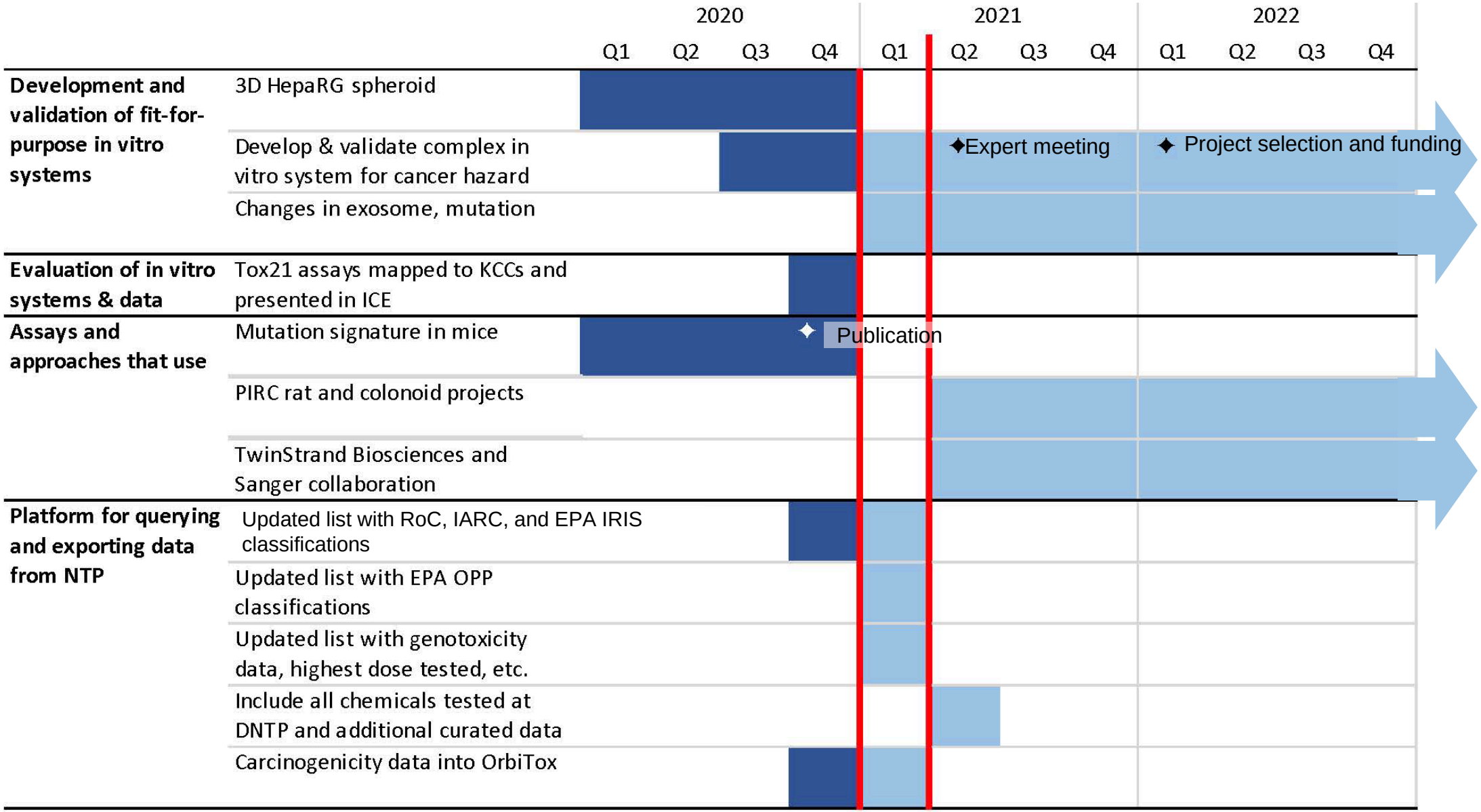
Stakeholders

Stakeholder Type

- Partner
- Collaborator
- Contributor
- User



Timeline of selected projects



Thank you!!