# Advances in Understanding Cancer Evolution and Cellular Response to Radiation

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# Questions in low dose radiation research: *In search of radiation-damage biomarkers*

- **Large-scale measurement of genomic profiles**
  - *Regions that confer susceptibility to radiation effects*
  - *Genomic landscape of radiation-related cancers*
  - *Epigenetic alterations*
- **Apply genomic technologies to robust radiation epidemiology studies**
  - *Study design*
  - *Strong Dosimetric data- KEY*
- **Informatic Pipelines & Data Sharing**
  - *Costly & always evolving…..*
  - *”Runaway costs”*

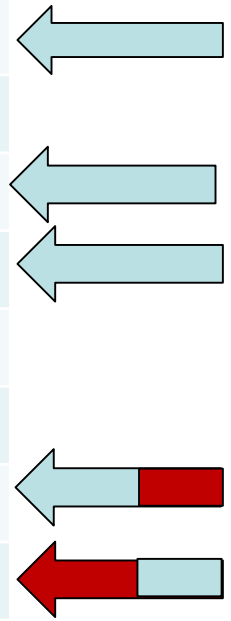
# Major Scientific Questions in Low Dose Radiation Research with Biospecimens

- **Germline/Normal Tissue**
  - Are there regions of the genome that protect or confer enhanced effects?
  - Possible field effects in adjacent/normal tissue
  - Possible effect on background mutational rates in different tissues (CH & mosaicism)
- **Somatic Alterations**
  - Reliable predictive biomarkers for radiation injury
    - Exposed individuals
    - Transgenerational setting
  - New insights into how radiation causes cancer & other conditions
    - Cancer Genome Landscapes- RNA & DNA
      - Signatures of low dose radiation exposure?
    - Clonal Hematopoiesis accelerated by radiation exposure
- **Epigenomic Events**
- **Circulating biomarkers of injury that predict risk**
  - Proteomics

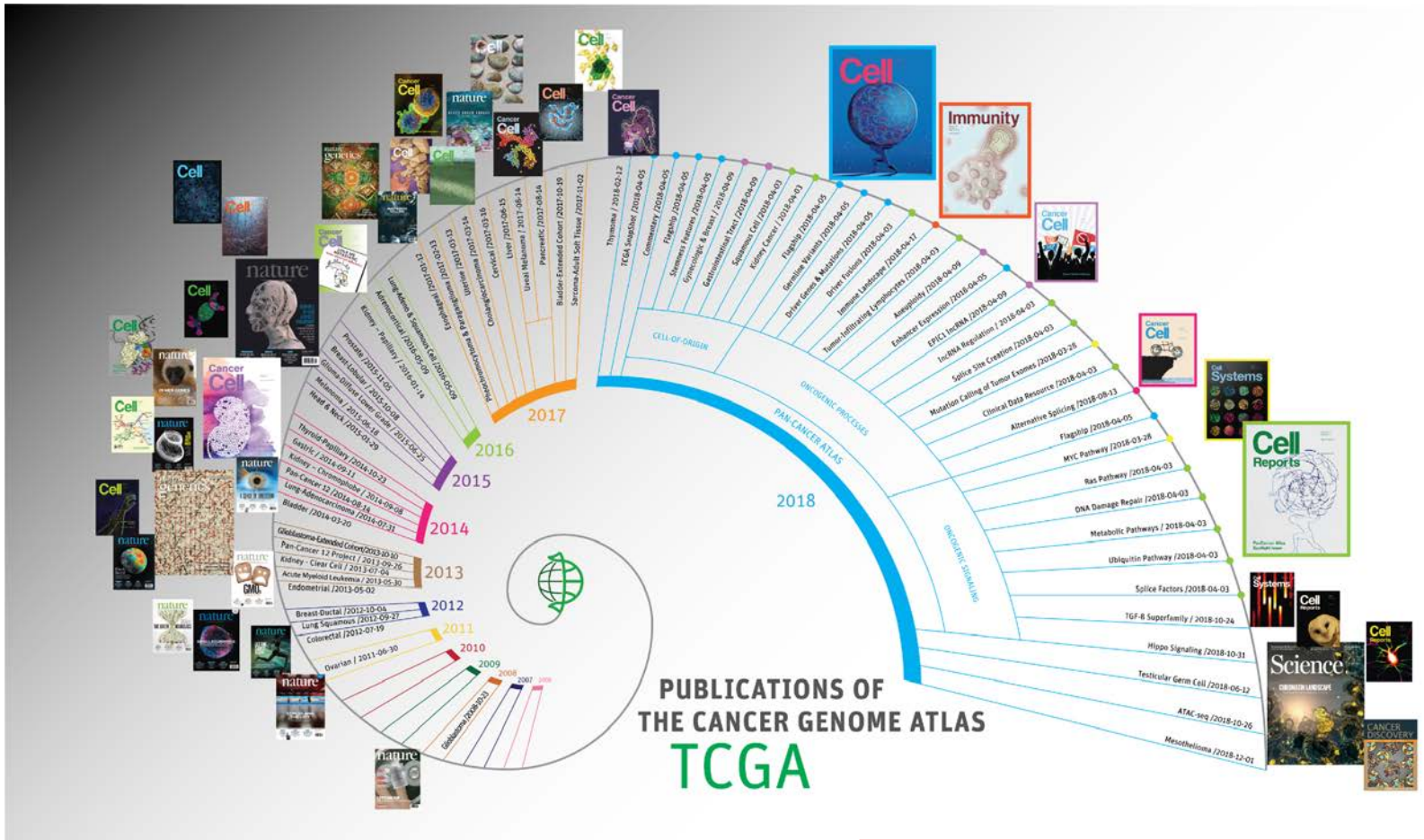
*In descending ease of large-scale analyses*

# Current REB/NCI Low-dose Radiation Research: Value of Well-Designed Epidemiologic Studies with Dosimetry

| Study | Key Collaborators |
|---|---|
| UK-NCI CT scans study | Univ of Newcastle |
| US Radiologic Technologists (USRT) | Univ of Minnesota |
| US interventional radiology physician cohort | AMA |
| Life Span Study | RERF |
| Ukrainian Trios Study | NRCRM |
| UkrAm (children & in utero) | IEM |
| BelAm (children & in utero) | RRCRM |
| Ukrainian liquidators case-control studies (thyroid/leukemia) | NRCRM |
| Chernobyl Tissue Bank | IEM |
| Low-dose pooling projects (leukemia/thyroid/brain cancer) | Multiple |
| UK Background radiation childhood cancer study | Univ of Oxford |
| Risk of Bias Assessment Methodology | IARC |



# Substantive Impact on Understanding Cancer: Genomic Landscape Analyses The Cancer Genome Atlas



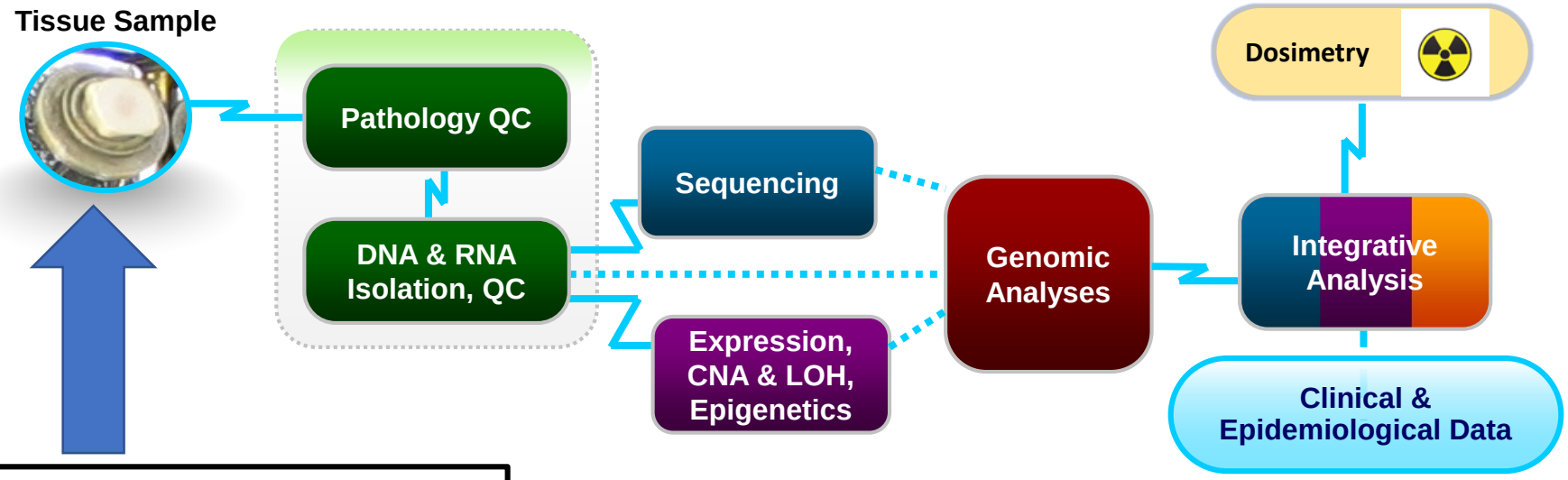

**Integrated Analyses**

*Pipelines in place*

1. DNA
   Germline & Somatic
2. Transcriptomic Profile
   mRNA & miRNA
3. Epigenetics
   focused CpG islands
4. Proteomics*
5. ***No collection of exposure***

Remarkable insights into biology of cancer, but….
Difficult to investigate causal effects of environmental exposures

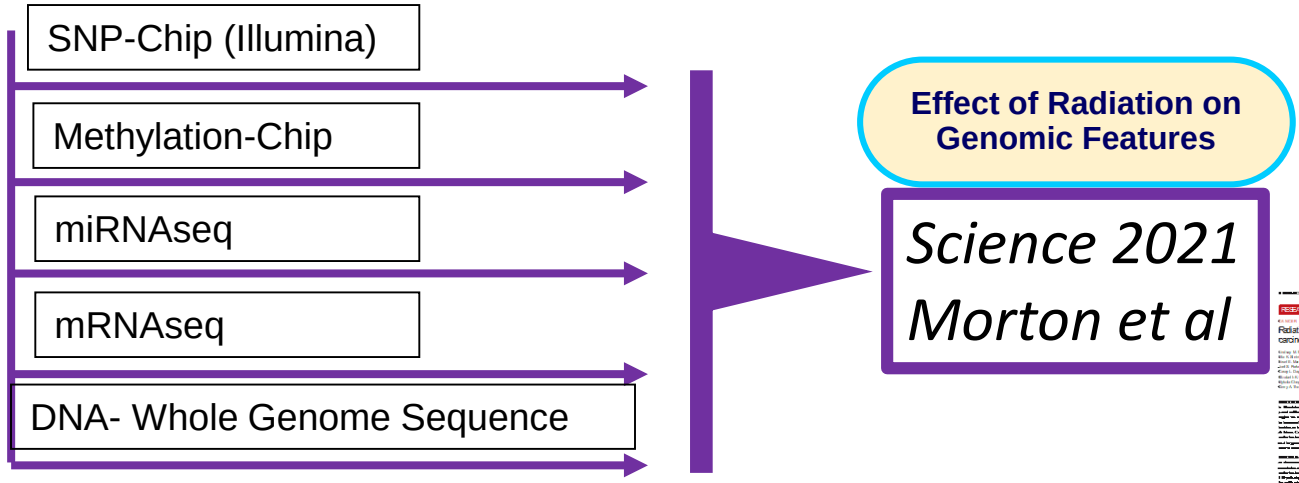
# Flow of Data for Chernobyl PTC Study: Available through the NCI Genomic Data Commons (genetics) & Chernobyl Tissue Bank (phenotypes)

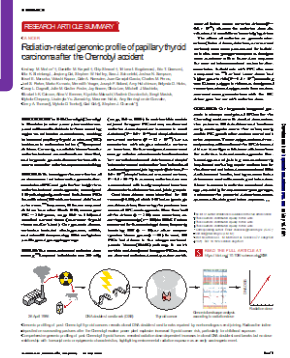



Chernobyl Tissue Bank (CTB)
- Gerry Thomas (PI)
- Panel review of Pathology

440 Confirmed Cases of PTC
- Fresh Frozen materials

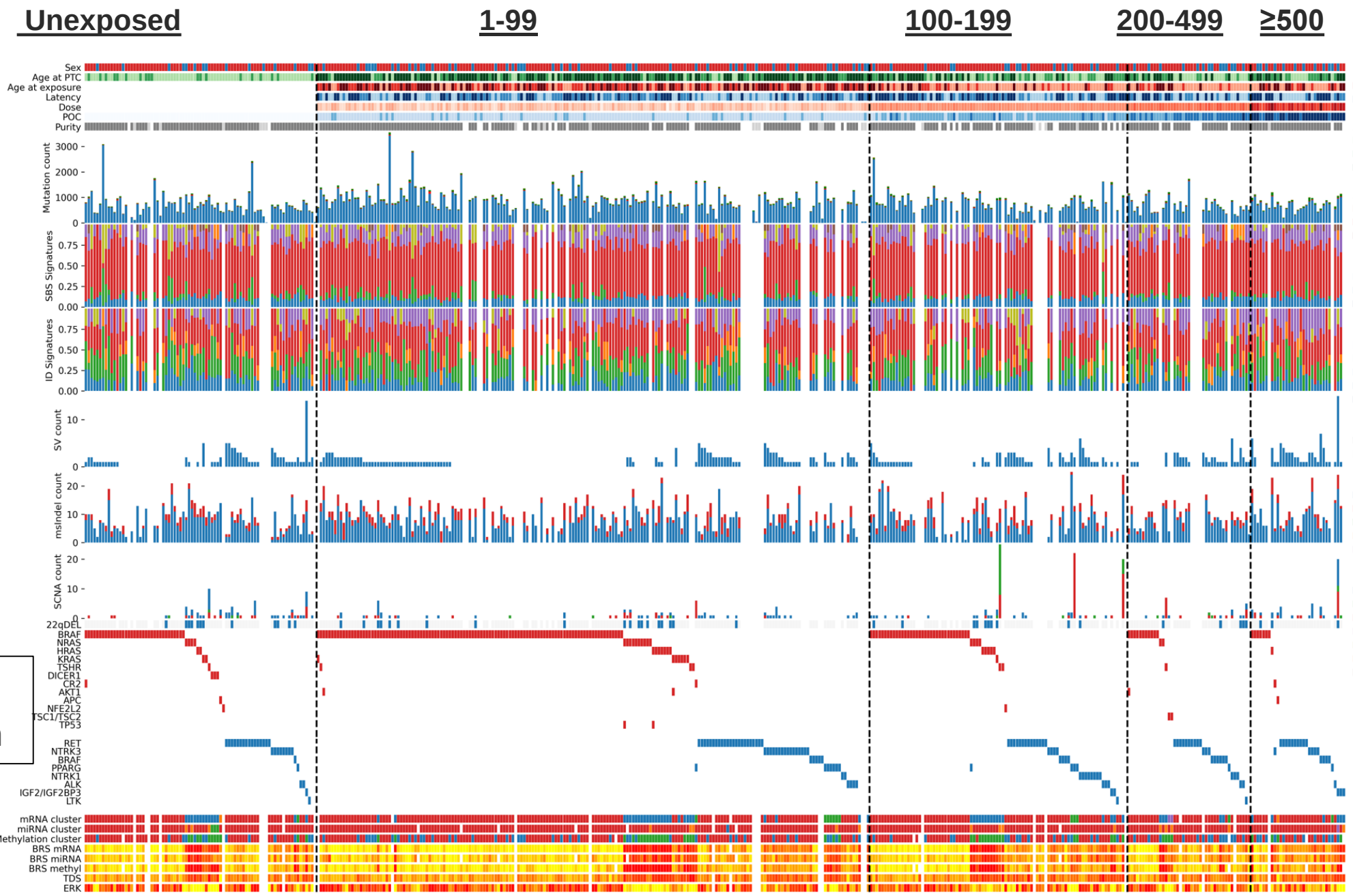
431 With matched normal tissue
- Non-tumor thyroid +/or Blood



**Genetic Data in GDC & Phenotypes in CTB**

# Landscape Plot of Genomic Alterations of Chernobyl PTC

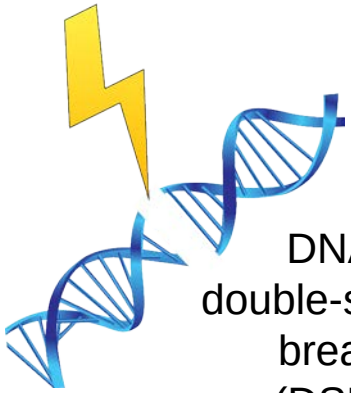



Driver Mutation

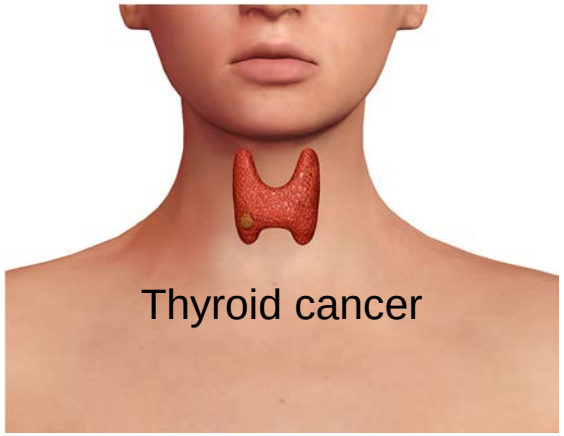
# Radiation Epidemiology & Genetics

April 26, 1986

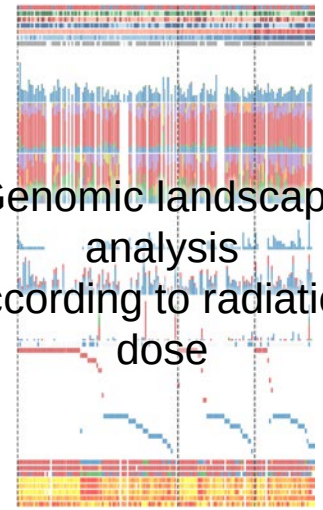




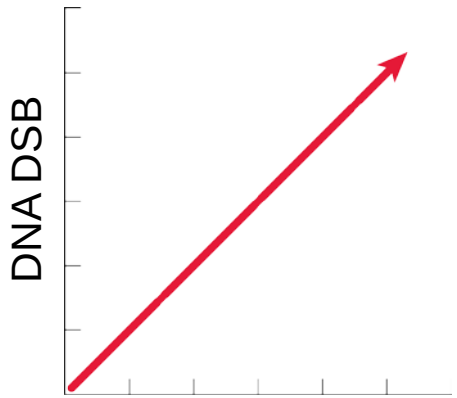
DNA double-strand break (DSB)

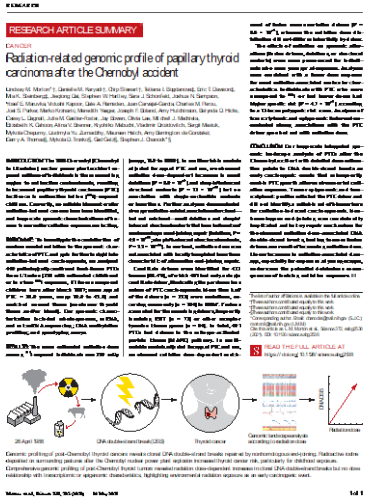


Thyroid cancer



Genomic landscape analysis according to radiation dose





RESEARCH ARTICLE SUMMARY

Radiation-related genomic profile of papillary thyroid carcinoma after the Chernobyl accident

*Morton et al Science 2021*

# Key Findings in Thyroid Tissue Analyses

1. >93% of driver mutations in MAPK pathway (ras/raf)
   - Radiation shifts towards fusion drivers
2. Non-homologous end joining DNA repair:
   - Fusions and structural variants
3. No field effect (radiation signature or drivers) in companion non-tumor thyroid
4. Epigenetic and gene expression profiles driven by cancer driver
   - No clear evidence for signatures of radiation effects

Morton et al. *Science* 2021

# Is There a Transgenerational Effect of Protracted Radiation Following Chernobyl Accident?

Approach: Determine de novo mutations in adult children born to

- Liquidators & Evacuees

- Family Structure Design
- Current Analysis
  - 105 families
  - 130 adult children
    - Born 1987-2002
  - High Coverage Whole Genome Sequencing
    - Short read
    - 80 X coverage *(higher than usual)*
- Multistep calling algorithm
  - Manual inspection of all putative events

American Journal of Epidemiology
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Advance Access publication:
July 2, 2020

Study Design

**Field Study of the Possible Effect of Parental Irradiation on the Germline of Children Born to Cleanup Workers and Evacuees of the Chornobyl Nuclear Accident**

**Dimitry Bazyka, Maureen Hatch, Natalia Gudzenko, Elizabeth K. Cahoon, Vladimir Drozdovitch, Mark P. Little, Vadim Chumak, Elena Bakhanova, David Belyi, Victor Kryuchkov, Ivan Golovanov, Kiyohiko Mabuchi, Iryna Ilienko, Yuri Belayev, Clara Bodelon, Mitchell J. Machiela, Amy Hutchinson, Meredith Yeager, Amy Berrington de Gonzalez, and Stephen J. Chanock***

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Although transgenerational effects of exposure to ionizing radiation have long been a concern, human research to date has been confined to studies of disease phenotypes in groups exposed to high doses and high dose rates, such as the Japanese atomic bomb survivors. Transgenerational effects of parental irradiation can be addressed using powerful new genomic technologies. In collaboration with the Ukrainian National Research Center for Radiation Medicine, the US National Cancer Institute, in 2014–2018, initiated a genomic alterations study among children born in selected regions of Ukraine to cleanup workers and/or evacuees exposed to low–dose-rate radiation after the 1986 Chornobyl (Chernobyl) nuclear accident. To investigate whether parental radiation exposure is associated with germline mutations and genomic alterations in the offspring, we are collecting biospecimens from father-mother-offspring constellations to study de novo mutations, minisatellite mutations, copy-number changes, structural variants, genomic insertions and deletions, methylation profiles, and telomere length. Genomic alterations are being examined in relation to parental gonadal dose, reconstructed using questionnaire and measurement data. Subjects are being recruited in exposure categories that will allow examination of parental origin, duration, and timing of exposure in relation to conception. Here we describe the study methodology and recruitment results and provide descriptive information on the first 150 families (mother-father-child(ren)) enrolled.

Chornobyl (Chernobyl); genetic risk; germline mutations; low–dose-rate radiation; parent-offspring constellations; preconception exposure; Ukraine

Abbreviations: DOB, date of birth; NCI, National Cancer Institute; NRCRM, National Research Center for Radiation Medicine.

Identifying the full extent of ionizing radiation–related health effects is critical for handling of nuclear accidents, radiation protection standards, and clinical practice. Pioneering experimental studies of transgenerational effects were carried out in 1927 (1) and 1958 (2), but until recently human research on transgenerational radiation effects following parental exposure has been limited. Sievert, in a 1958 paper prepared for the International Commission on Radiological Protection (3), pointed to the scarcity of data on possible genetic effects of radiation in humans. Sixty years later, this remains an important and controversial issue, particularly in the wake of the Chornobyl (Chernobyl) and Fukushima nuclear power plant accidents, which occurred in 1986 and 2011, respectively. Intervening studies have been confined to studies of disease phenotypes and minisatellite mutation rates in Japanese atomic bomb survivors, Chornobyl-exposed groups, and offspring of childhood cancer survivors, mostly exposed to high doses/rates (4). With the





Bazyka et al. *Amer J Epi* 2020

# Long-standing Controversy on Possible Transgenerational Effect Due to Radiation

## Prior Animal Data

- High doses (2-4 Gy) acute exposures tested
- Evidence of double strand breaks
  - NHEJ DNA Repair
- Structural and chromosomal events
  - No single base mutational signature

## Prior Human Studies

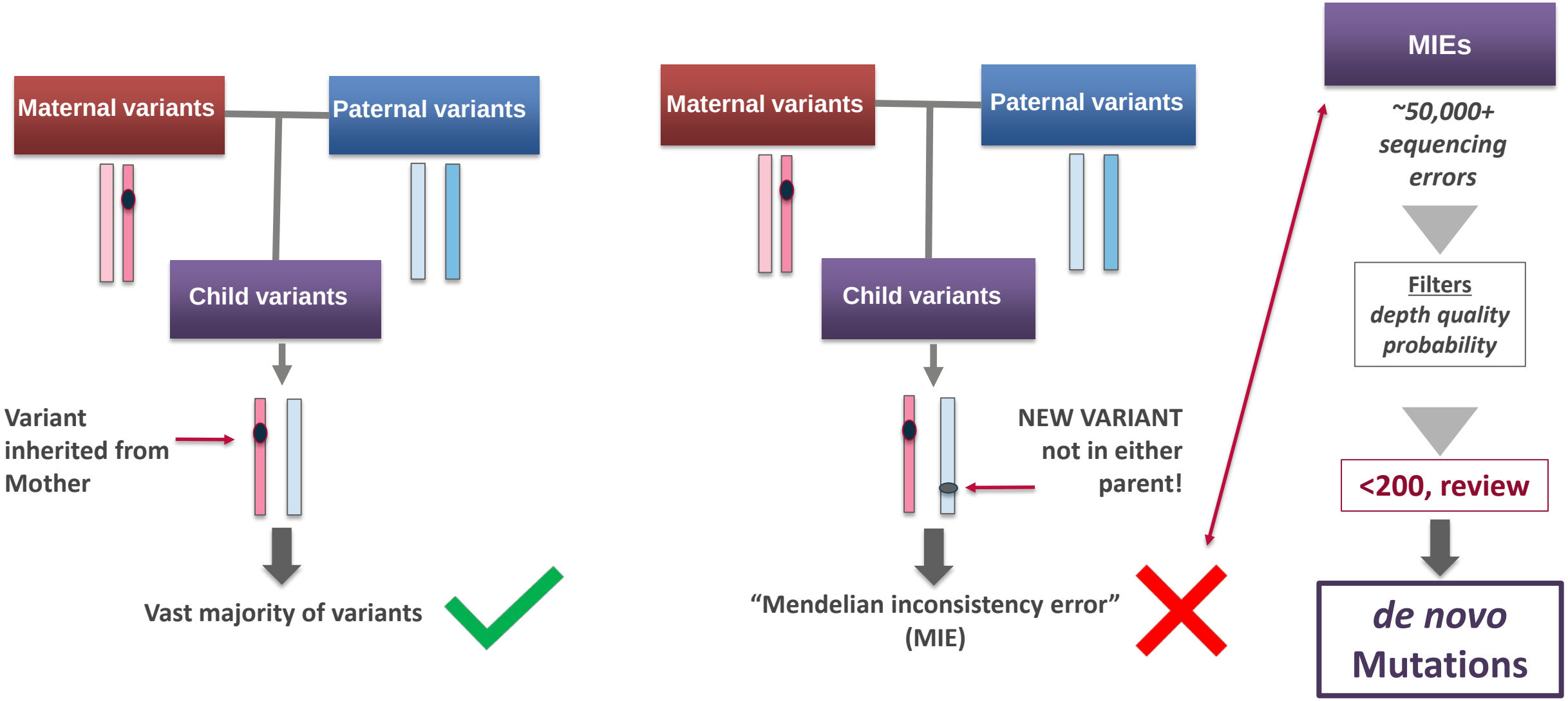
- Lower/protracted doses
- Investigation of outcomes
- Small, underpowered studies of genetic markers
  - Microsatellites (too few)
- Nagaski study of three family trios (whole genome sequence)
  - Anecdotal

# *An essential type of mutation……*
# *De Novo Mutations (DNMs)*

- Due to random mutations in gametes (sperm and oocytes), a small number of variants are generated
- Transmitted variants are known as de novo mutations (DNMs)
- Critical blocks of evolution
- Only class of genomic variation to NOT undergo purifying selection
- DNMs account for a fraction of neurodevelopmental disorders
- DNMs ascertained by analysis of both parents with child(ren)
  - “Seen” only in child but not parents

# Principle for Detecting *De Novo* Mutations (DNMs)

***Family Trio Whole Genome Sequencing***



# Associations of Age at Conception, Cumulative Ionizing Radiation Dose, and Smoking History with Total DNM Count

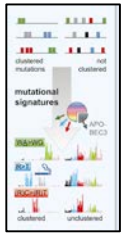
| | Estimate | 95% Confidence Interval | P-value |
|---|---|---|---|
| **Age at conception** | | | |
| Maternal age | 0.46 | -0.02, 0.93 | 0.06 |
| Paternal age | 1.94 | 1.51, 2.36 | $3.65\times10^{-15}$ |
| **Cumulative radiation dose (/mGy)** | | | |
| Maternal dose | -0.02 | -0.04, 0.007 | 0.17 |
| Paternal dose | -0.0007 | -0.003, 0.002 | 0.56 |
| **Smoking history** | | | |
| Maternal former smoker | -4.13 | -10.74, 2.49 | 0.22 |
| Maternal current smoker | 5.31 | -0.18, 10.81 | 0.06 |
| Paternal former smoker | 0.91 | -5.16, 6.97 | 0.77 |
| Paternal current smoker | 2.91 | -0.93, 6.75 | 0.14 |

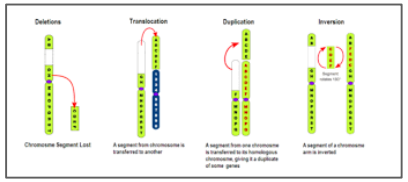
Yeager et al. *Science* 2021

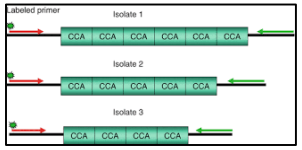
# Important Considerations

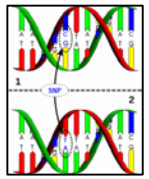
- Risk of small change of DNMs is very small (if at all)
- Timing of radiation dose could be critical
- Further trio studies of higher dose exposure
  - Chernobyl
  - Survivors of Hiroshima/Nagasaki (RERF)
- Not clear it is worthwhile for lower doses (Fukushima)
- Clonal Hematopoiesis (CH) in Liquidators
  - Increased CH seen following radiotherapy
  - Higher coverage of select genes (*DNMT3A, TET2, TP53, etc*)

# Effect estimates of cumulative radiation dose on subgroups of DNMs*




| | Estimate | 95% Confidence Interval | P-value |
|---|---|---|---|
| Clusters (n = 181) | | | |
| Paternal Dose (mGy) | -0.0002 | -0.0005, 0.0002 | 0.33 |
| Maternal Dose (mGy) | 0.003 | -0.0008, 0.006 | 0.13 |
| Complex (n = 50) | | | |
| Paternal Dose (mGy) | 0.00001 | -0.0002, 0.0002 | 0.91 |
| Maternal Dose (mGy) | -0.0003 | -0.002, 0.002 | 0.73 |
| Indels (n = 2,103) | | | |
| Paternal Dose (mGy) | 0.0002 | -0.001, 0.001 | 0.78 |
| Maternal Dose (mGy) | -0.01 | -0.02, 0.002 | 0.12 |
| Microsatellites (n = 730) | | | |
| Paternal Dose (mGy) | 0.0003 | -0.0004, 0.001 | 0.41 |
| Maternal Dose (mGy) | -0.002 | -0.008, 0.004 | 0.51 |
| SNVs (n = 9,388) | | | |
| Paternal Dose (mGy) | -0.0008 | -0.003, 0.002 | 0.51 |
| Maternal Dose (mGy) | -0.01 | -0.03, 0.01 | 0.39 |
| Total DNMs (n = 11,722) | | | |
| Paternal Dose (mGy) | -0.0007 | -0.003, 0.002 | 0.56 |
| Maternal Dose (mGy) | -0.02 | -0.04, 0.007 | 0.17 |

*Microsatellites are a further subgrouping of Indels & adjusted for sequencing batch, parental age, and parental smoking status

# Challenge of Subtype Events Analyses: Statistical Power & Read Length

**Current reports based on 'short read' NGS (150-300 bp)**

**Types of events:**

- Complex & Cluster events are very rare ~1-2 per generation
- Microsatellites ~8-10 per generation
- Detection of transgenerational radiation effect
  - Power estimates require substantially larger trio sets

**Future opportunity- Long read NGS (Pacbio or Oxford Nanopore)**

- Read lengths in 1-15 kb enable rare complex events to be identified
- Input DNA- enormous (can not use WGA-amplified DNA)

# *Resource considerations for well-designed studies*: ‘Agnostic’ genome-wide vs candidate studies

1. Input material varies
   - Commercial Arrays (SNP/epigenetics) 50 ng
   - Short read NGS 100-200 ng
   - Long read NGS 10 ug
2. Analytic pipelines
   - Ready for ‘agnostic’ genome wide analyses
     - Short read DNA & RNA
     - SNP/methylation chip analsyes
   - Not ready for agnostic genome wide analyses
     - Long range NGS (PacBio & ONP)
     - Epigenomic (bisulfite whole genome sequencing)

# “The GeNOME”
# “Friend or foe?”

Promising

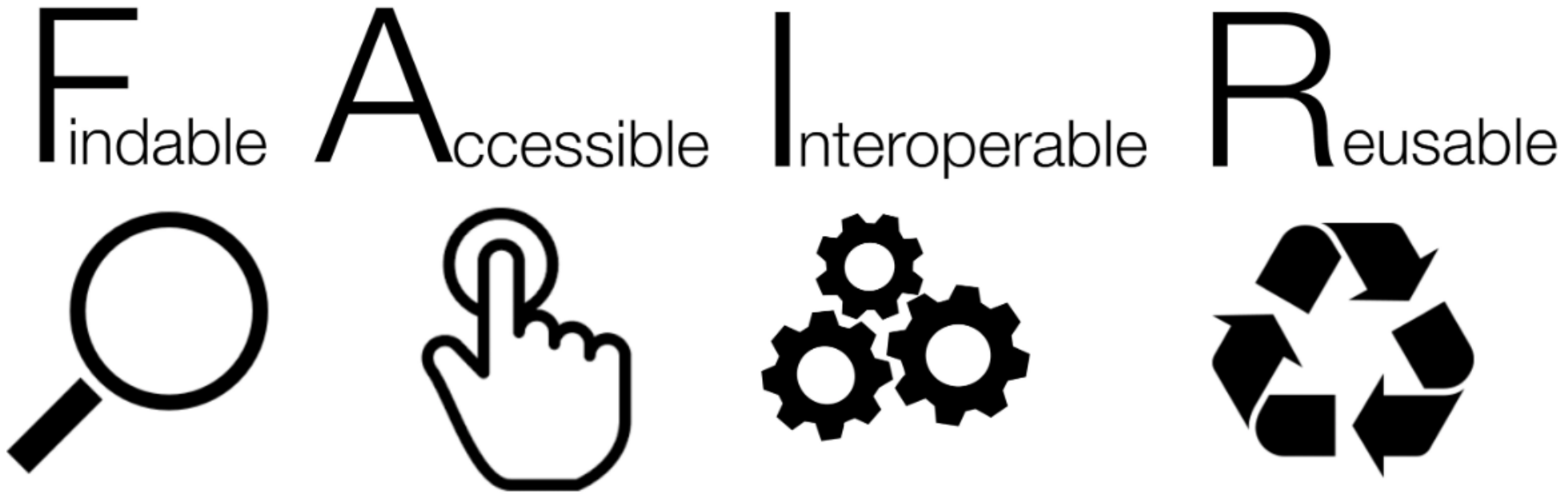
Haunting


Dangerous

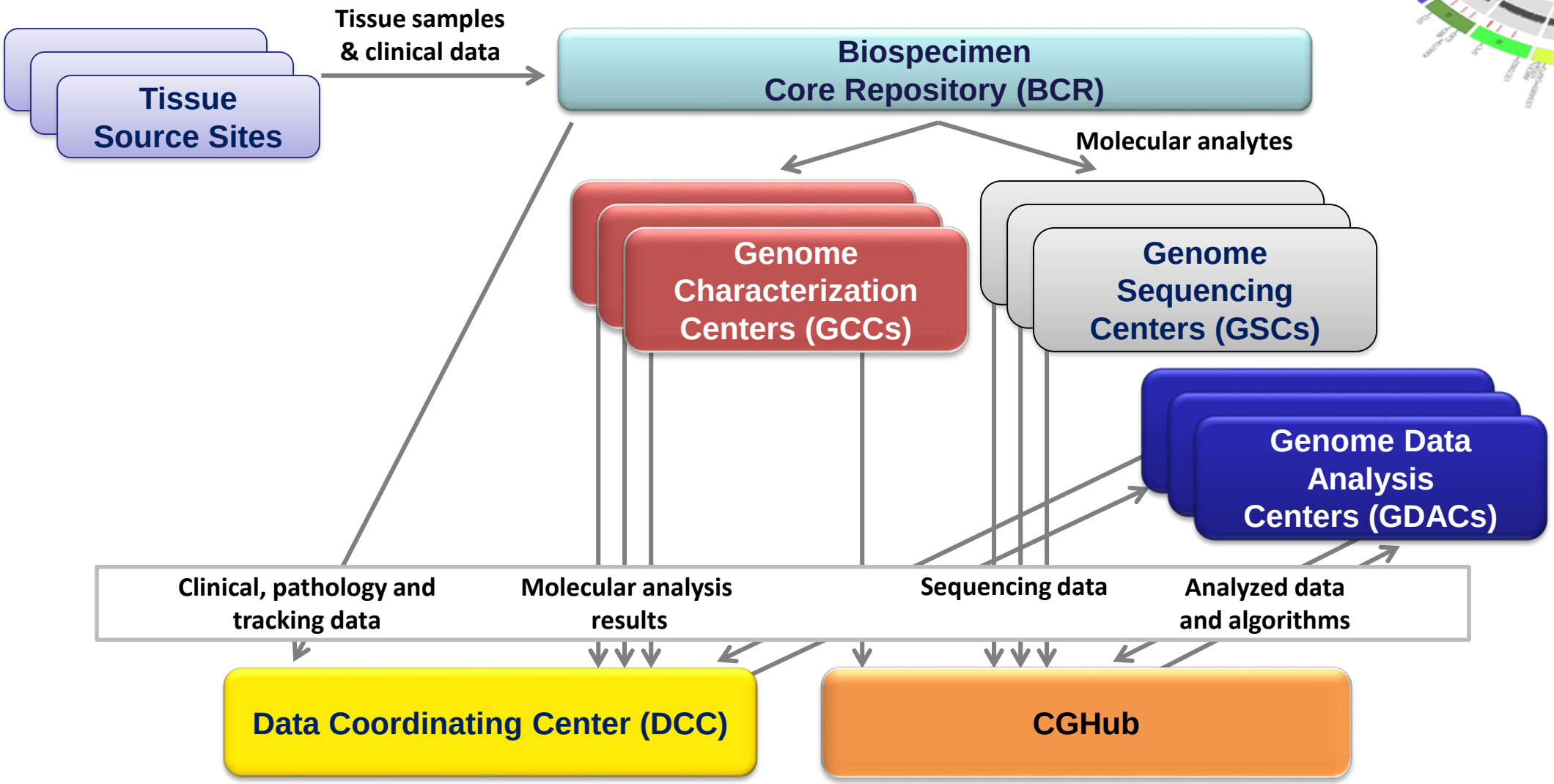
# Informatics and Analyses: Team Science High Cost for Personnel and IT Structures

- High performance computational capabilities
  - Containerized analytical programs
  - Large cluster availability or
  - Cloud Computing
- Large scale storage
  - Movement of data
- Team of experts to analyze data
- Team of experts to manage data
- Team of experts to interpret data

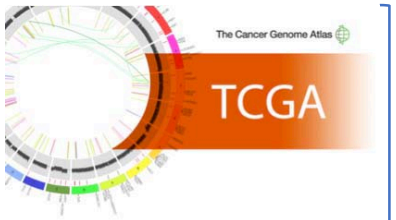
# FAIR data management
# FAIR data sharing



# TCGA Data Flow for “omics” (but lacking epidemiological & clinical data)



# NCI Genomic Data Commons (GDC) Functionality







*User-submitted studies*


Genomic/ clinical data →


NCI Genomic Data Commons

1. Import and standardize genomic and clinical data from NCI programs
2. Harmonize mapping of sequence data to the most current genome
3. Implement state-of-art analytic methods:
   - mutation calls
   - copy number
   - structural variants
   - digital gene expression
4. Maintain data security and manage authorized access
5. Enable data browsing, download and on-site analysis
6. Open GDC for upload of new cancer genomic data from researchers worldwide for data analysis and and sharing.

# GDC Supported Programs

## NCI Programs

THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute


HCMI
Human Cancer Models Initiative

CTSP (Clinical Trial Sequencing Project)

ER (Exceptional Responders)

CDDP (Cancer Driver Discovery Program)

ALCHEMIST
ERLOTINIB TREATMENT TRIAL (A081105)

CLINICAL PROTEOMIC TUMOR ANALYSIS CONSORTIUM

## Non-NCI Programs

AACR
American Association for Cancer Research
FINDING CURES TOGETHER™

PROJECTGENIE
Genomics Evidence Neoplasia Information Exchange

FOUNDATION MEDICINE®

American Society of Hematology
Helping hematologists conquer blood diseases worldwide

APOLLO Network

LEUKEMIA & LYMPHOMA SOCIETY®

Beat AML
acute myeloid leukemia

MMRF
MULTIPLE MYELOMA Research Foundation

Count Me In

# Recommendations:

- **Hybrid System for Funding**
  - Managed project at large scale: Costs are high for ‘omics’
  - Centers for specific analyses using publicly available data (GDC)
  - RFAs for projects to use large-scale data resource and pursue specific aims
    - Immense value of investigative biology
- **Rationale for Hybrid System**
  - Upfront Costs for Major Project(s)
    - Study designs & dosimetry
    - Generation of data for integration
  - Avoid a series of underpowered, inadequate studies
  - Support functional follow-up based on integration of data
- **Modern data sharing policies & use of cloud-technologies**
  - Best way to engage international community

# Questions?


ONE PROGRAM,
MANY PEOPLE,
INFINITE POSSIBILITIES

irp.nih.gov