The key characteristics approach to hazard identification

Martyn Smith

School of Public Health,

University of California, Berkeley CA, USA

UC BERKELEY
SUPERFUND
RESEARCH PROGRAM
SCIENCE FOR A SAFER WORLD

martynts@berkeley.edu

http://superfund.berkeley.edu

Summary of today's talk

- Scientific findings providing insights into cancer mechanisms play an increasingly important role in carcinogen hazard identification
- **The key characteristics (KCs) of human carcinogens provide the basis for a knowledge-based approach to evaluating mechanistic data rather than a hypothesis-based one like MOA/AOP**
- Recent IARC Monograph, EPA, CalEPA and NTP evaluations have illustrated the applicability of the KC approach
- May be compatible with HT assays, but need to develop new ones based on characteristics and hallmarks. Same for biomarkers.
- Key characteristics for other forms of toxicity are being developed
- KCs could be used in data-science approach to prioritorize chemicals for further evaluation

Integration of evidence to decide if a chemical is a human carcinogen?

- Human studies – epidemiology
- Animal studies – usually rodent bioassays – lifetime chronic or shorter transgenic assays?
- In vitro studies – e.g. Tox21/Toxcast
- Mechanistic data – Provides biological plausibility and increasing in importance

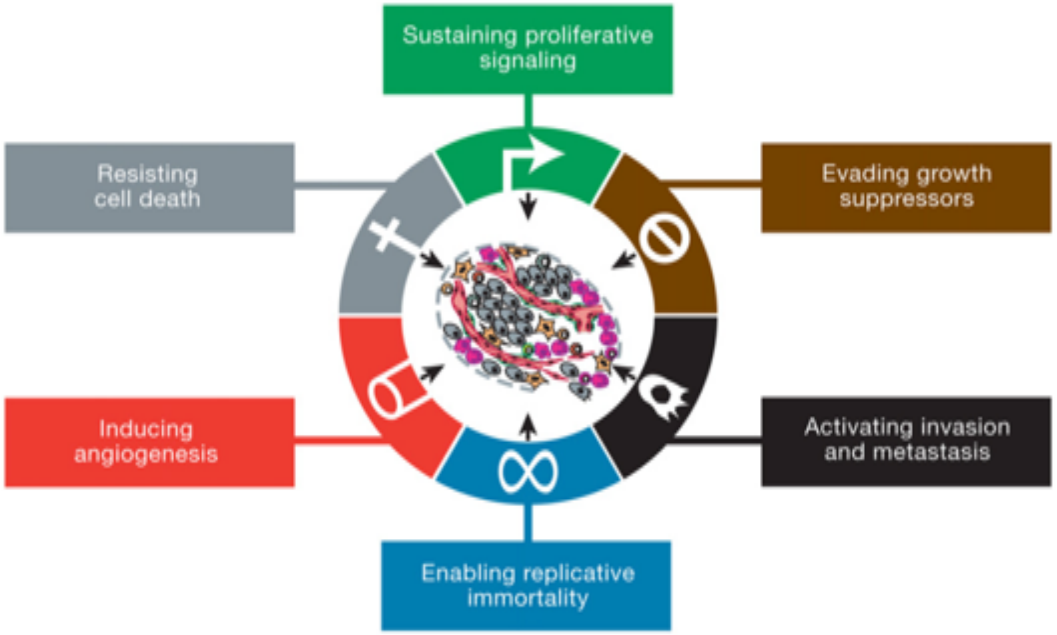
Mechanistic Data: *Challenges*

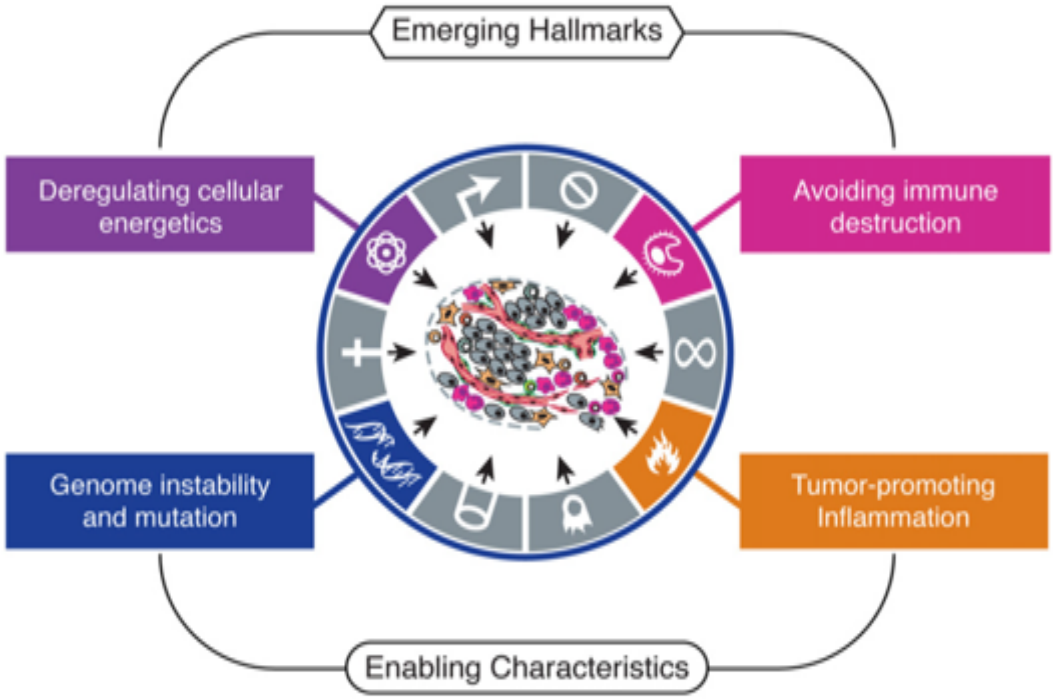
IARC Monographs
Volume 100

- How to search systematically for relevant mechanisms?
- How to bring uniformity across assessments?
- How to analyze the voluminous mechanistic database efficiently?
- How to avoid bias towards favored mechanisms?

KCs resulted from a large collaboration

- **IARC**: Kathryn Z. Guyton, Robert Baan and Kurt Straif
- **US EPA**: Catherine Gibbons, Jason Fritz, David DeMarini, Jane Caldwell, Robert Kavlock, Vincent Cogliano
- **NTP**: John Bucher **FDA**: Frederick Beland
- **Academia**: Ivan Rusyn, Paul F. Lambert, Stephen S. Hecht, Bernard W. Stewart, Weihsueh Chiu, Denis Corpet, Martin van den Berg, Matthew Ross, David Christiani
- **Consultant**: Christopher Portier
- **Acknowledgements:** Michele La Merrill and others for discussion and support from Lauren Zeise of OEHHA and Research Translation Core of NIEHS SRP grant P42ES004705.





HALLMARKS OF CANCER

1. Sustaining proliferative signaling
2. Evading growth suppressors
3. Resisting cell death
4. Enabling replicative immortality
5. Inducing aberrant angiogenesis
6. Activating invasion & metastasis

Emerging Hallmarks

- Reprogramming energy metabolism
- Evading immune destruction

Enabling Characteristics

- Genomic instability and mutation
- Inflammation

Hanahan and Weinberg 2011

Chemicals disrupt multiple hallmarks

Kleinstreuer N.C. et al. In vitro perturbations of targets in cancer hallmark processes predict rodent chemical carcinogenesis. Toxicol. Sci., (2013) 131, 40–55.

Chemical	HM1	HM2	HM3	HM4	HM5	HM6	HM7	HM8	HM9	HM 10	**TOTAL**
Chemical 1	X	X			X			X	X	X	**7**
Chemical 2			X	X			X				**3**
Chemical 3					X			X			**2**
Chemical 4	X	X		X			X	X	X		**6**

Tested 292 chemicals in 672 assays and successfully correlated the most disruptive chemicals (i.e. those that were most active across the various hallmarks) with known levels of carcinogenicity.

Multiple Mechanisms of Group 1 Carcinogens

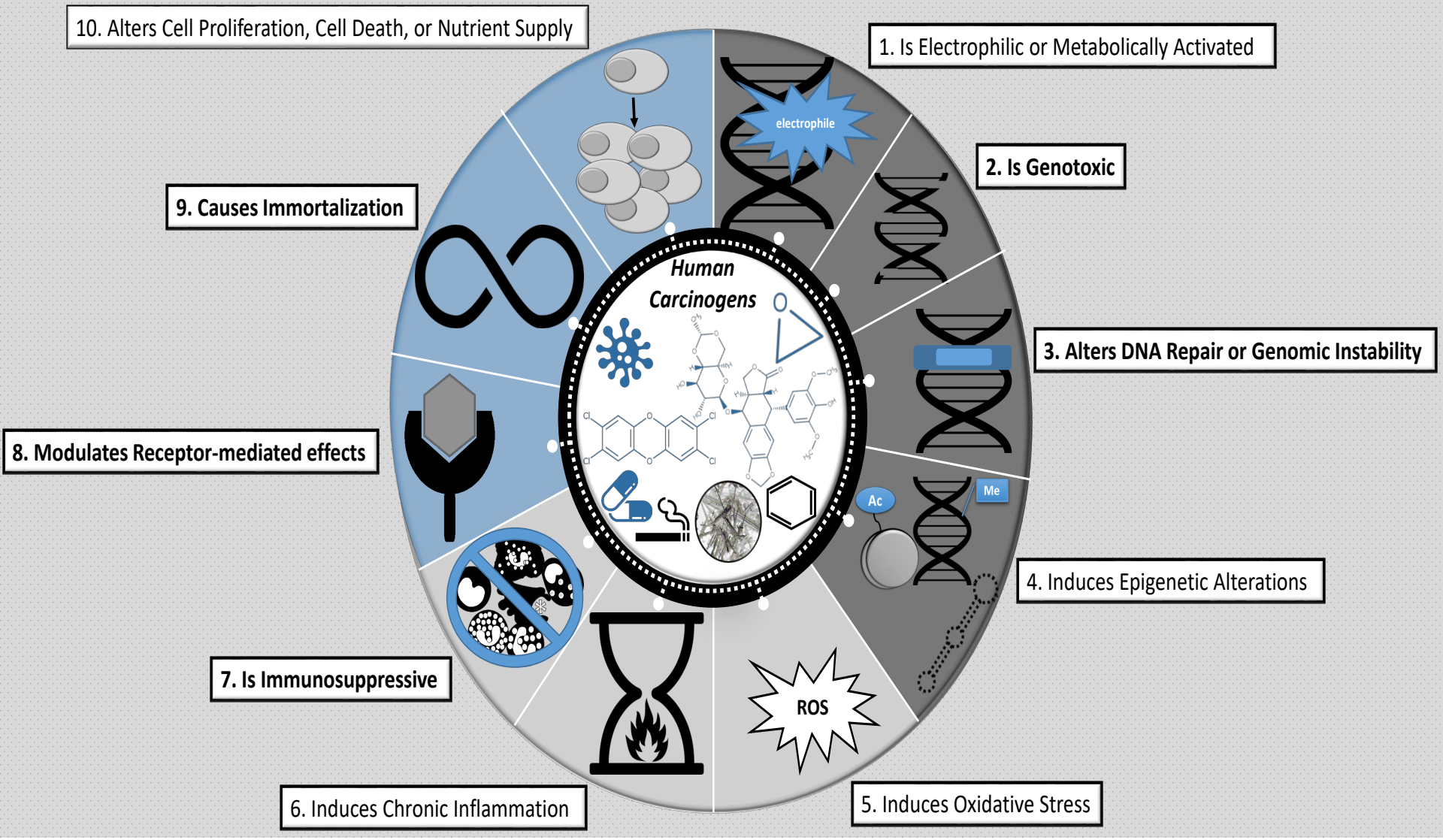
[KZ Guyton….MT Smith, Mut Res 681; 230, 2009]

	Carcinogen			
Mechanisms	**Aflatoxin B1**	**Arsenic**	**Asbestos**	**Benzene**
DNA damage	+	+	-	+
Gene mutation	+	-	+	-
Chrom mutation	+	+	+	+
Aneuploidy	-	+	+	+
Epigenetic	+	+		+
Receptor signaling	-	+	+	
Other signaling	-	+		+
Immune effects	+	+	+	+
Inflammation	+	+	+	+
Cytotoxicity	+	+	+	+
Mitogenic	-	+		-
Gap junction	+	+		+

Dilemma: Cancer or Carcinogens

- Hallmarks are the biological characteristics of cancer cells and tumors in general, NOT the characteristic properties of human carcinogens
- Need to identify the key characteristics of human carcinogens
- IARC Working Group did this in 2012 and subsequently scientists at EPA, IARC and elsewhere determined how these characteristics could be searched for systematically

THE KEY CHARACTERISTICS OF HUMAN CARCINOGENS



Guyton KZ, Rieswijk L, et al., Chemical Res. In Toxicology, December 6, 2018

10 Key Characteristics of Human Carcinogens

Key characteristic:
1. Is electrophilic or can be metabolically activated
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

Smith MT, Guyton KZ, Gibbons CF, Fritz JM et al.. *Env Health Persp.*, *124(6):713-21*

- **Established human carcinogens** commonly exhibit one or more characteristics
- Data on these characteristics can **provide evidence of carcinogenicity**
- They can also **help in interpreting** the relevance and importance of findings of cancer in animals and in humans.

Characteristic	Examples of relevant evidence
1. Is Electrophilic or Can Be Metabolically Activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.
2. Is Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces Epigenetic Alterations	DNA methylation, histone modification, microRNA expression
5. Induces Oxidative Stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)

Characteristic	Examples of relevant evidence
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is Immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes Immortalization	Inhibition of senescence, cell transformation, altered telomeres
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

A Hallmark *versus* a Key Characteristic

- A Hallmark describes what is *(biology)*
- A Key Characteristic (KC) of a *chemical* describes a property that makes the "what is" happen

MT Smith, UCB April 2019

Critical, Early Event

EXPOSED CELL in BODY

CARCNOGENS

KEY CHARACTERISTICS (KCs)

KC 1,2 Electrophilicity, Genotoxicity

KC 3 Altered DNA repair, Genomic Instability

KC4 Epigenetic changes

KC 5 Oxidative stress

KC 6 Inflammation

KC 8 Receptor-mediated effects

KC 10 Cell proliferation

KC 10 Inhibit apoptosis

KC 9 Immortalization

KC 10 Nutrient supply

KC 7 Immunosuppression

KC 5 Oxidative stress

KC 6 Inflammation

DNA damage, Mutations, Epigenetic changes

CANCER CELL

HALLMARKS

Genomic instability and mutation

Sustaining proliferative signaling

Evading growth suppressors

Resisting cell death

Enabling replicative immortality

Inducing aberrant angiogenesis

Evading immune destruction

Reprogramming energy metabolism

Activating invasion & metastasis

Inflammation

According to Kansas City native Bill Goodson the KCs were bound to integrate with the Hallmarks

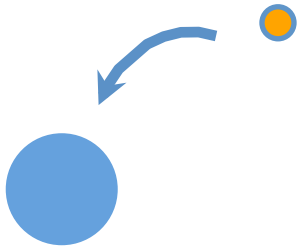
Exception: KC of Sunshine Band fame is from Florida

Applications of the KCs

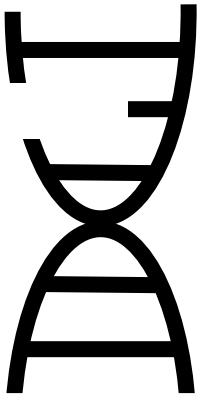
- Searching the literature – Set of MeSH terms developed – Facilitate systematic review
- Identify data gaps
- Development of MOA/AOP or networks
- Improve predictive toxicology
- Better understanding of cumulative risk

Strong Evidence of 5 Key Characteristics for Sb^{III}

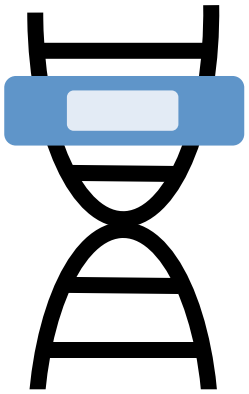
Electrophilic



Genotoxic

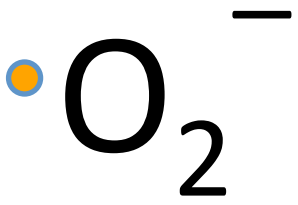


↓ DNA repair



→Epigenetic alteration

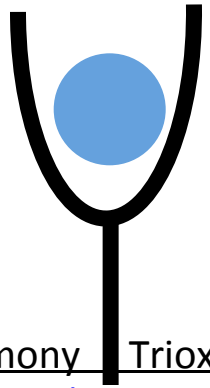
↑ Oxidative stress



→Chronic inflammation

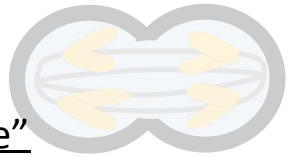
↑↓ Immune response

→ Receptor-mediated effects



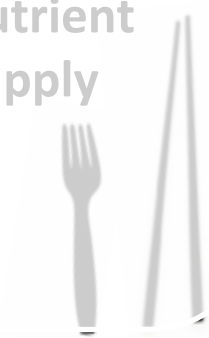
→Cell immortalization

↑Cell proliferation,



↓cell death,

or alter nutrient supply



"Report on Carcinogens Monograph on Antimony Trioxide"
https://ntp.niehs.nih.gov/pubhealth/roc/listings/antimonyt/index.html

Applications of the KCs

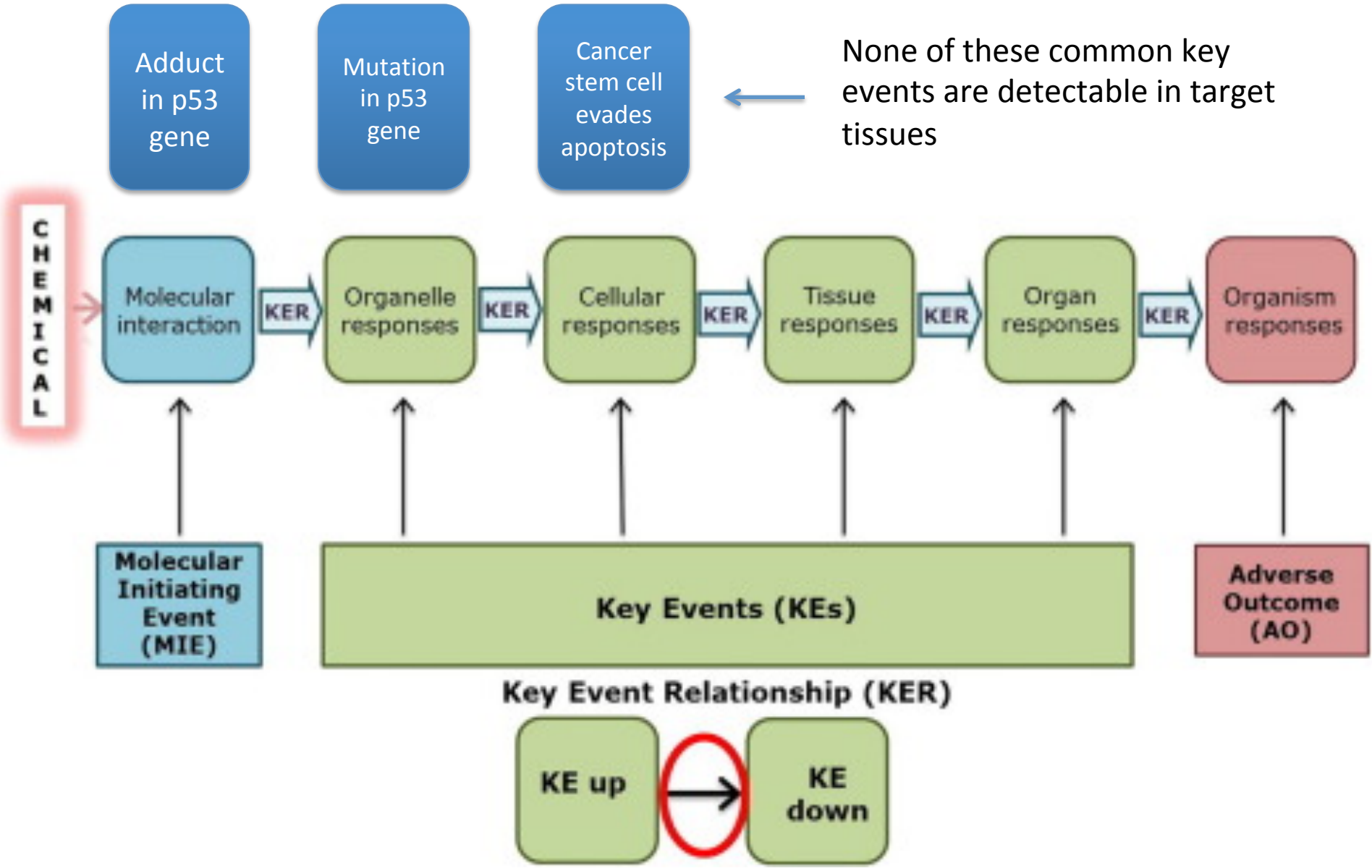
- Searching the literature – Set of MeSH terms developed – Facilitate systematic review
- Identify data gaps
- Development of MOA/AOP or networks
- Improve predictive toxicology
- Better understanding of cumulative risk

Limitations of MOA/AOP Approach

- Biology is not linear – influenced by feedback mechanisms, repair, background, susceptibilities… Network of systems
- Multiple ways to arrive at same conclusion – Does not fit with Causal Pie concept
- Limited by the current understanding of the disease process (recognized by Sir Bradford Hill, who noted that “what is biologically plausible depends upon the biological knowledge of the day”)
- Key events are supposed to be quantifiable but in reality they may be impossible to measure

Limitations of MOA/AOP Approach

- Biology is not linear – influenced by feedback mechanisms, repair, background, susceptibilities… Network of systems
- Multiple ways to arrive at same conclusion – Does not fit with Causal Pie concept
- Limited by the current understanding of the disease process (recognized by Sir Bradford Hill, who noted that "what is biologically plausible depends upon the biological knowledge of the day")
- Key events are supposed to be quantifiable but in reality they may be impossible to measure



Key Events in a Carcinogenic AOP

MT Smith, UCB April 2019

Limitations of MOA/AOP Approach (continued)

- Focus on ‘favorite’ mechanism may introduce bias, especially on committees and public databases
- MOA/AOP may be incomplete or wrong [e.g. DEHP – Rusyn and Corton (2012)]
- How many ‘validated’ AOPs needed for 100K chemicals producing 100s of adverse outcomes in different ways?

Key characteristics don't require risk assessor to guess the mechanism

- Mechanistic hypotheses in science are beneficial because if you test it and are wrong then you modify the hypothesis and get closer to the truth

- Mechanistic hypotheses in risk assessment are problematic because if you are wrong you may have made a bad risk decision that cannot easily be changed and may have caused medical or economic harm

National Academy of Sciences report released January 5, 2017

https://www.nap.edu/download/24635

Using 21st Century Science to Improve Risk-Related Evaluations

260 pages | 6 x 9 | PAPERBACK
ISBN 978-0-309-45348-6 | DOI: 10.17226/24635

AUTHORS

Committee on Incorporating 21st Century Science into Risk-Based Evaluations; Board on Environmental Studies and Toxicology; Division on Earth and Life Studies; National Academies of Sciences, Engineering, and Medicine

Using 21st Century Science to Improve Risk-Related Evaluations - Comments

- The KC "approach avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence." (P.144)
- "The committee notes that key characteristics for other hazards, such as cardiovascular and reproductive toxicity, could be developed as a guide for evaluating the relationship between perturbations observed in assays, their potential to pose a hazard, and their contribution to risk." (p.141)
- Through a project funded by OEHHA (Cal EPA), KCs for male and female reproductive toxicants and endocrine disruptors have been developed and KCs for cardio- and neuro-toxicants will be developed next

Working Group on KCs of Endocrine Disruptors and Reproductive Toxicants

Berkeley CA, March 7-8, 2018

What Next for the Key Characteristics?

- Refinement of definitions and listing of all assays for each characteristic
- Development of HT assays specific for each characteristic – A CarciCAST – Testing of new drugs and chemicals (see Fielden et al. 2018)
- Key characteristics of other endpoints – cardiovascular toxicity; developmental neurotoxicity etc.

Question for the Future

Can we predict that a chemical possesses multiple key characteristics using HTS/ toxicogenomic data and prioritize it for further evaluation as a possible/probable human carcinogen?

Summary

- Scientific findings providing insights into cancer mechanisms play an increasingly important role in carcinogen hazard identification
- **The key characteristics of known human carcinogens provide the basis for a knowledge-based approach to evaluating mechanistic data rather than a hypothesis-based one like MOA/AOP**
- Shows carcinogens tend to act through multiple mechanisms in producing the hallmarks of human and animal tumors
- Recent IARC Monograph, EPA, CalEPA and NTP evaluations have illustrated the applicability of the KC approach
- May be compatible with HT assays, but need to develop new ones based on characteristics and hallmarks. Same for biomarkers.
- Key characteristics for other forms of toxicity are being developed


UC BERKELEY
SUPERFUND
RESEARCH PROGRAM
SCIENCE FOR A SAFER WORLD
Thank you for listening!