Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis

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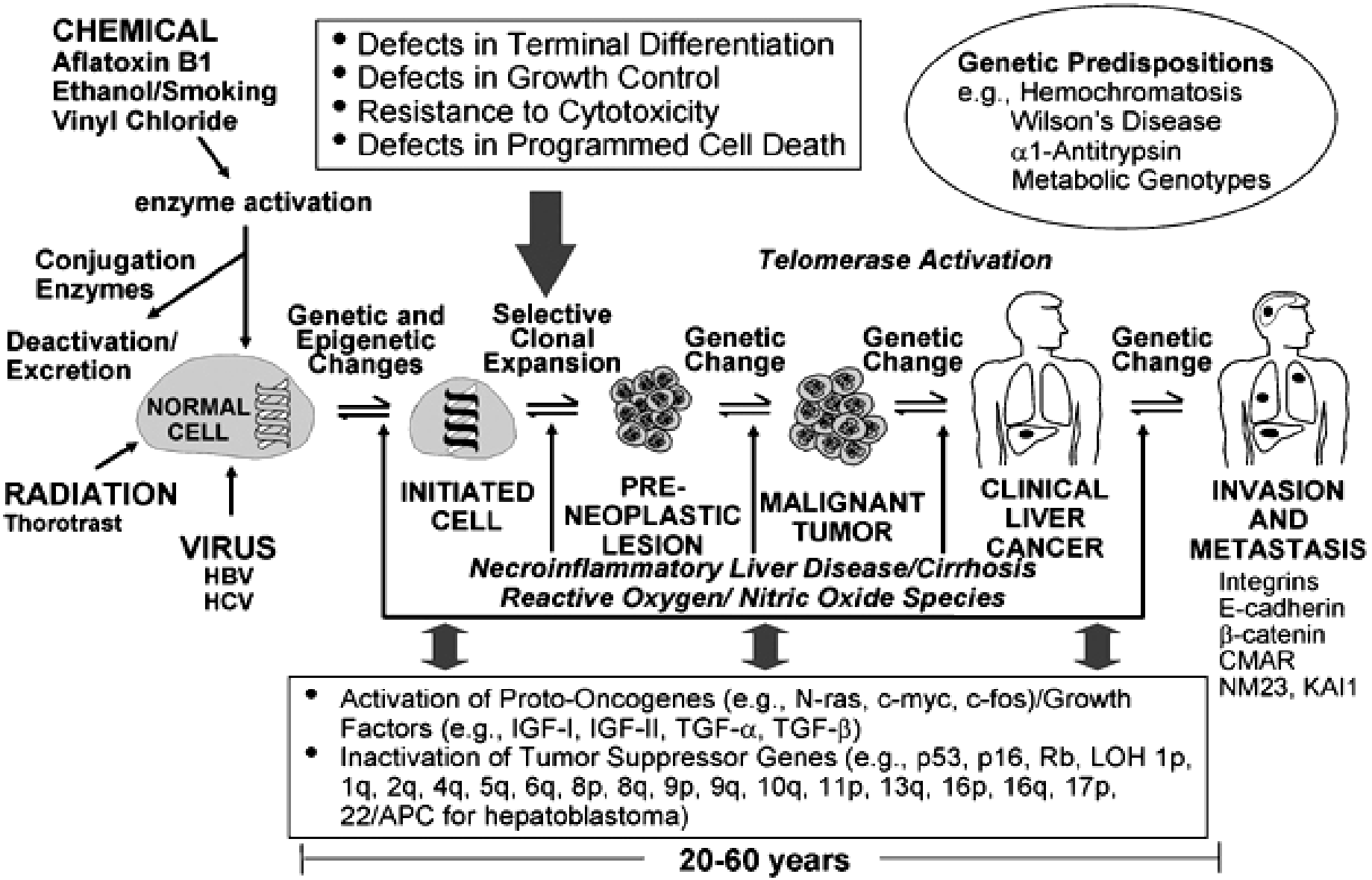
Mechanistic data - Problems to address

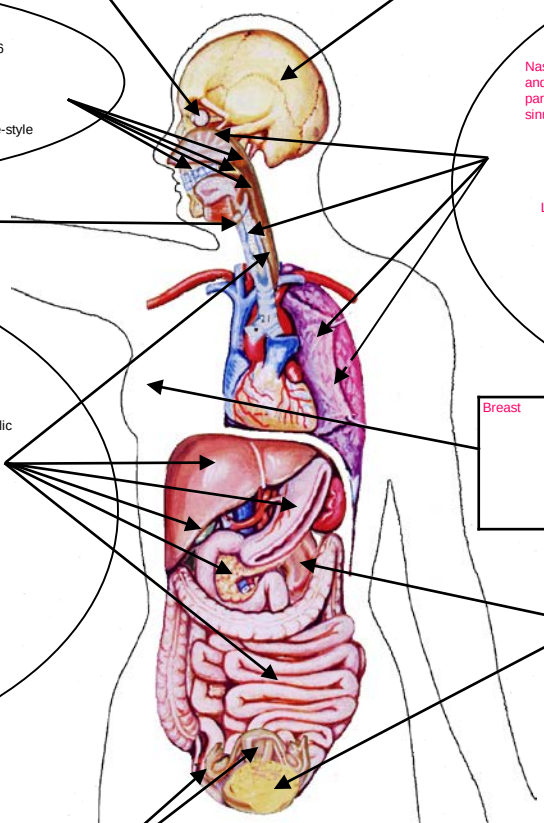
- There is no broadly accepted, systematic method for identifying, organizing, and summarizing mechanistic data for the purpose of decision-making in cancer hazard identification
- Many human carcinogens act via multiple mechanisms causing various biological changes in the multistage process of carcinogenesis – How to capture these diverse effects that lead to cancer and other adverse outcomes for all types of agents?

Human Tumors and Stages of Carcinogenesis



Hussain *et al.,* Oncogene, 2007

IARC Monographs Volume 100: The known causes of human cancer by organ site



Eye
Human immunodeficiency virus type 1
Ultraviolet-emitting tanning devices
Welding

Brain and central nervous system
X-radiation, gamma-radiation

Oral cavity
Alcoholic beverages
Betel quid with tobacco
Betel quid without tobacco
Human papillomavirus type 16
Smokeless tobacco
Tobacco smoking

Pharynx
Alcoholic beverages
Betel quid with tobacco
Human papillomavirus type 16
Tobacco smoking

Nasopharynx
Epstein-Barr virus
Formaldehyde
Salted fish, Chinese-style
Wood dust

Tonsil
Human papillomavirus type 16

Salivary gland
X-radiation, gamma-radiation

Thyroid
Radioiodines, including iodine-131 (exposure during childhood and adolescence)
X-radiation, gamma-radiation

Lung
Aluminium production
Arsenic and inorganic arsenic compounds
Asbestos (all forms)
Beryllium and beryllium compounds
Bis(chloromethyl)ether; chloromethyl methyl ether (technical grade)
Cadmium and cadmium compounds
Chromium (VI) compounds
Coal, indoor emissions from household combustion
Coal gasification
Coal-tar pitch
Coke production
Haematite mining (underground)
Iron and steel founding
MOPP (vincristine-prednisone-nitrogen mustard-procarbazine mixture)
Nickel compounds
Painter (occupational exposure as)
Plutonium
Radon-222 and its decay products
Rubber production industry
Silica dust, crystalline
Soot
Sulfur mustard
Tobacco smoke, secondhand
Tobacco smoking
X-radiation, gamma-radiation

Nasal cavity and paranasal sinus
Isopropyl alcohol manufacture using strong acids
Leather dust
Nickel compounds
Radium-226 and its decay products
Radium-228 and its decay products
Tobacco smoking
Wood dust

Larynx
Acid mists, strong inorganic
Alcoholic beverages
Asbestos (all forms)
Tobacco smoking

Mesothelioma (pleura or peritoneum)
Asbestos (all forms)
Erionite
Painter (occupational exposure as)

Stomach
Helicobacter pylori
Rubber production industry
Tobacco smoking
X-radiation, gamma-radiation

Upper aerodigestive tract
Acetaldehyde associated with consumption of alcoholic beverages

Liver (hepatocytes)
Aflatoxins
Alcoholic beverages
Estrogen-progestogen contraceptives
Hepatitis B virus
Hepatitis C virus
Plutonium
Thorium-232 and its decay products
Tobacco smoking (in smokers and in smokers' children)
Vinyl chloride

Oesophagus
Acetaldehyde associated with consumption of alcoholic beverages
Alcoholic beverages
Betel quid with tobacco
Betel quid without tobacco
Smokeless tobacco
Tobacco smoking
X-radiation, gamma-radiation

Gall bladder
Thorium-232 and its decay products

Biliary tract
Chlonorchis sinensis
Opisthorchis viverrini

Pancreas
Smokeless tobacco
Tobacco smoking

Colon and rectum
Alcoholic beverages
Tobacco smoking
X-radiation, gamma-radiation

Anus
Human immunodeficiency virus type 1
Human papillomavirus type 16

Breast
Alcoholic beverages
Diethylstilbestrol
Estrogen-progestogen contraceptives
Estrogen-progestogen menopausal therapy
X-radiation, gamma-radiation

Urinary bladder
Aluminium production
4-Aminobiphenyl
Arsenic and inorganic arsenic compounds
Auramine production
Benzidine
Chlornaphazine
Cyclophosphamide
Magenta production
2-Naphthylamine
Painter (occupational exposure as)
Rubber production industry
Schistosoma haematobium
Tobacco smoking
ortho-Toluidine
X-radiation, gamma-radiation

Kidney
Tobacco smoking
X-radiation, gamma-radiation

Renal pelvis and ureter
Aristolochic acid, plants containing
Phenacetin
Phenacetin, analgesic mixtures containing
Tobacco smoking

Endometrium
Estrogen menopausal therapy
Estrogen-progestogen menopausal therapy
Tamoxifen

Uterine cervix
Diethylstilbestrol (exposure *in utero*)
Estrogen-progestogen contraceptives
Human immunodeficiency virus type 1
Human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Tobacco smoking

Vagina
Diethylstilbestrol (exposure *in utero*)
Human papillomavirus type 16

Vulva
Human papillomavirus type 16

Ovary
Asbestos (all forms)
Estrogen menopausal therapy
Tobacco smoking

Penis
Human papillomavirus type 16

Multiple sites (unspecified)
Cyclosporine
Fission products, including Strontium-90
X-radiation, gamma-radiation (exposure *in utero*)

All cancers combined
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin

Bone
Plutonium
Radium-224 and its decay products
Radium-226 and its decay products
Radium-228 and its decay products
X-radiation, gamma-radiation

Endothelium (Kaposi sarcoma)
Human immunodeficiency virus type 1
Kaposi sarcoma herpes virus

Group 1 agents with less than *sufficient* evidence in humans
2,3,4,7,8-Pentachlorodibenzofuran
3,4,5,3',4'-Pentachlorobiphenyl (PCB-126)
4,4'-Methylenebis(2-chloroaniline) (MOCA)
Alpha- and beta-particle emitters
Areca nut
Aristolochic acid
Benzidine, dyes metabolised to
Benzo[*a*]pyrene
Ethanol in alcoholic beverages
Ethylene oxide
Etoposide
Ionizing radiation (all types)
Neutron radiation
N'-Nitrosonornicotine (NNN) and 4-(*N*-nitroso-methylamino)-1-(3-pyridyl)-1-butanone (NNK)

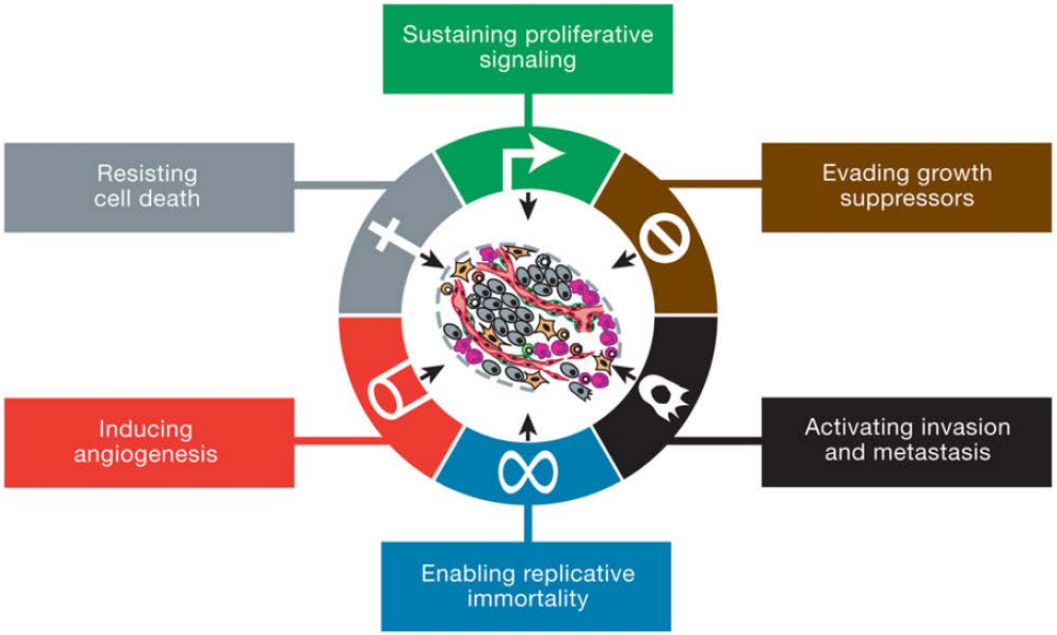
Skin (melanoma)
Solar radiation
Ultraviolet-emitting tanning devices

Skin (other malignant neoplasms)
Arsenic and inorganic arsenic compounds
Azathioprine
Coal-tar distillation
Coal-tar pitch
Cyclosporine
Methoxsalen plus ultraviolet A
Mineral oils, untreated or mildly treated
Shale oils
Solar radiation
Soot
X-radiation, gamma-radiation

Leukaemia/lymphoma
Azathioprine
Benzene
Busulfan
1,3-Butadiene
Chlorambucil
Cyclophosphamide
Cyclosporine
Epstein-Barr virus
Etoposide with cisplatin and bleomycin
Fission products, including Strontium-90
Formaldehyde
Helicobacter Pylori
Hepatitis C virus
Human immunodeficiency virus type 1
Human T-cell lymphotropic virus type 1
Kaposi sarcoma herpes virus
Melphalan
MOPP (vincristine-prednisone-nitrogen mustard-procarbazine mixture)
Phosphorus-32, as phosphate
Rubber production industry
Semustine [1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, or methyl-CCNU]
Thiotepa
Thorium-232 and its decay products
Tobacco smoking
Treosulfan
X-radiation, gamma-radiation

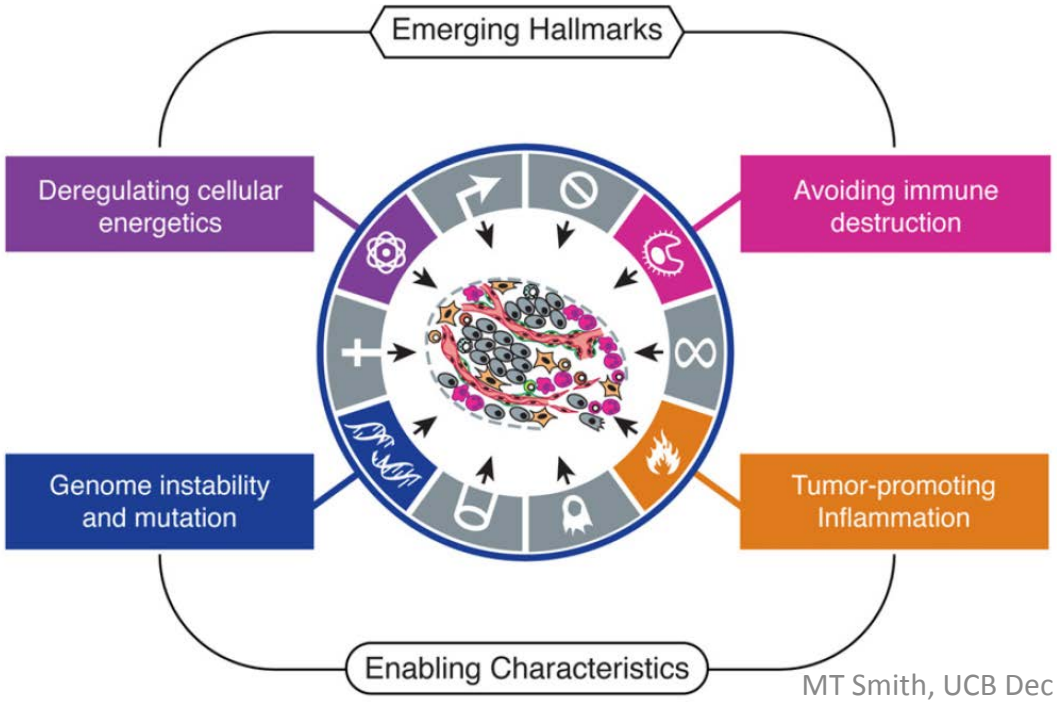
Section of the IARC Monographs (IMO)

Two meetings held at IARC in 2012 on concordance and mechanisms



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

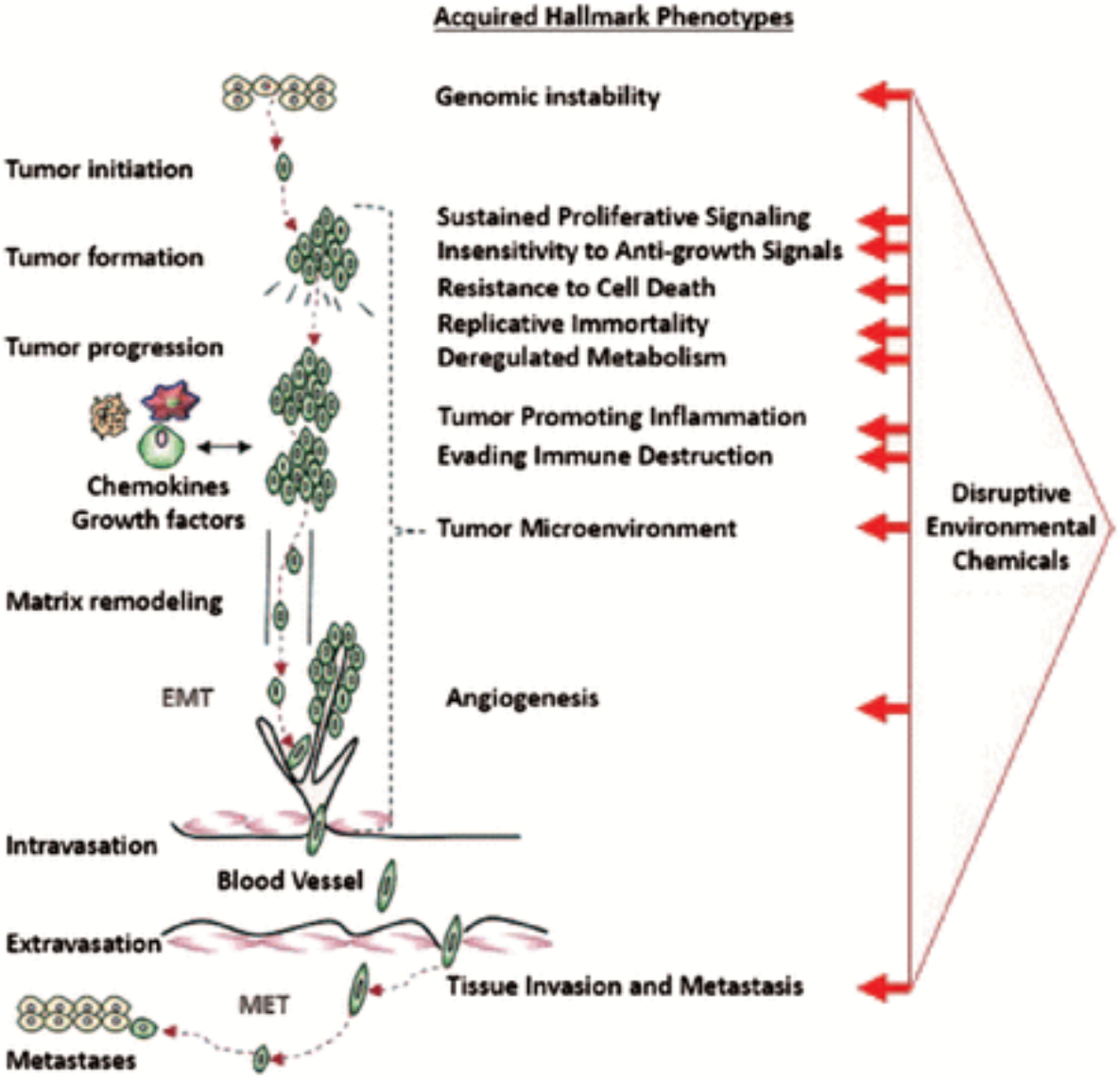
- Reprogramming energy metabolism
- Evading immune destruction

Enabling Characteristics

- Genomic instability and mutation
- Inflammation

Hanahan and Weinberg 2011

Chemicals and other stressors act at different points on the disease continuum



"Considering the multistep nature of cancer and the acquired capabilities implied by each of these hallmarks, it is therefore a very small step to envision how a series of complementary exposures acting in concert might prove to be far more carcinogenic than predictions related to any single exposure might suggest. Interacting contributors need not act simultaneously or continuously, they might act sequentially..."

Goodson et al. Carcinogenesis. 2015 Jun; 36(Suppl 1): S254–S296.

Review team	Chemical name	Disruptive action on key mechanism / pathway	Low-dose effect (LDE, LLDE, NLDE, threshold, unknown)
Angiogenesis	Diniconazole	Vascular cell adhesion molecule and cytokine signaling	Threshold (H-PC) (36 =TOXCAST)
	Chlorothalonil	Thrombomodulin, vascular proliferation and cytokine signaling	Unknown (H-PC) (36), NLDE (A-*in vivo*) (38 in Amphibians)
Immune system evasion	Pyridaben	Chemokine signaling, TGF-β, FAK, HIF-1a, IL-1a pathways	Unknown (H-CL, H-PC, A-CL) (36,139,140), threshold (A-I) (141)
	Triclosan	Chemokine signaling, TGF-β, FAK, IL-1a pathways	Threshold (H-CL, H-PC, A-I) (36,142–144), LDE (A-I, H-CL) (145,146) None of these papers (142-146) show immune evasion

Examples of endpoints used to support conclusions of Goodson et al. --

MT Smith, UCB Dec 2015

Problem is that assay endpoints don't match hallmarks

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

Multiple Mechanisms of IARC Group 1 Carcinogens

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

	Carcinogen			
Mechanisms	AFB1	As+3	Asbestos	Benzene
DNA damage	+	+	-	+
Gene mutation	+	-	+	-
Chrom mutation	+	+	+	+
Aneuploidy	-	+	+	+
Epigenetic	+	+		+
Receptor signaling	-	+	+	
Other signaling	-	+		+
Immune effects	+	+	+	+
Inflammation	+	+	+	+
Cytotoxicity	+	+	+	+
Mitogenic	-	+		-
Gap junction	+	+		+

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

Evidence that these characteristics are observed, especially in humans or as intermediate biomarkers in human specimens can provide biological plausibility for epidemiological findings and/or early warning if no epidemiology exists

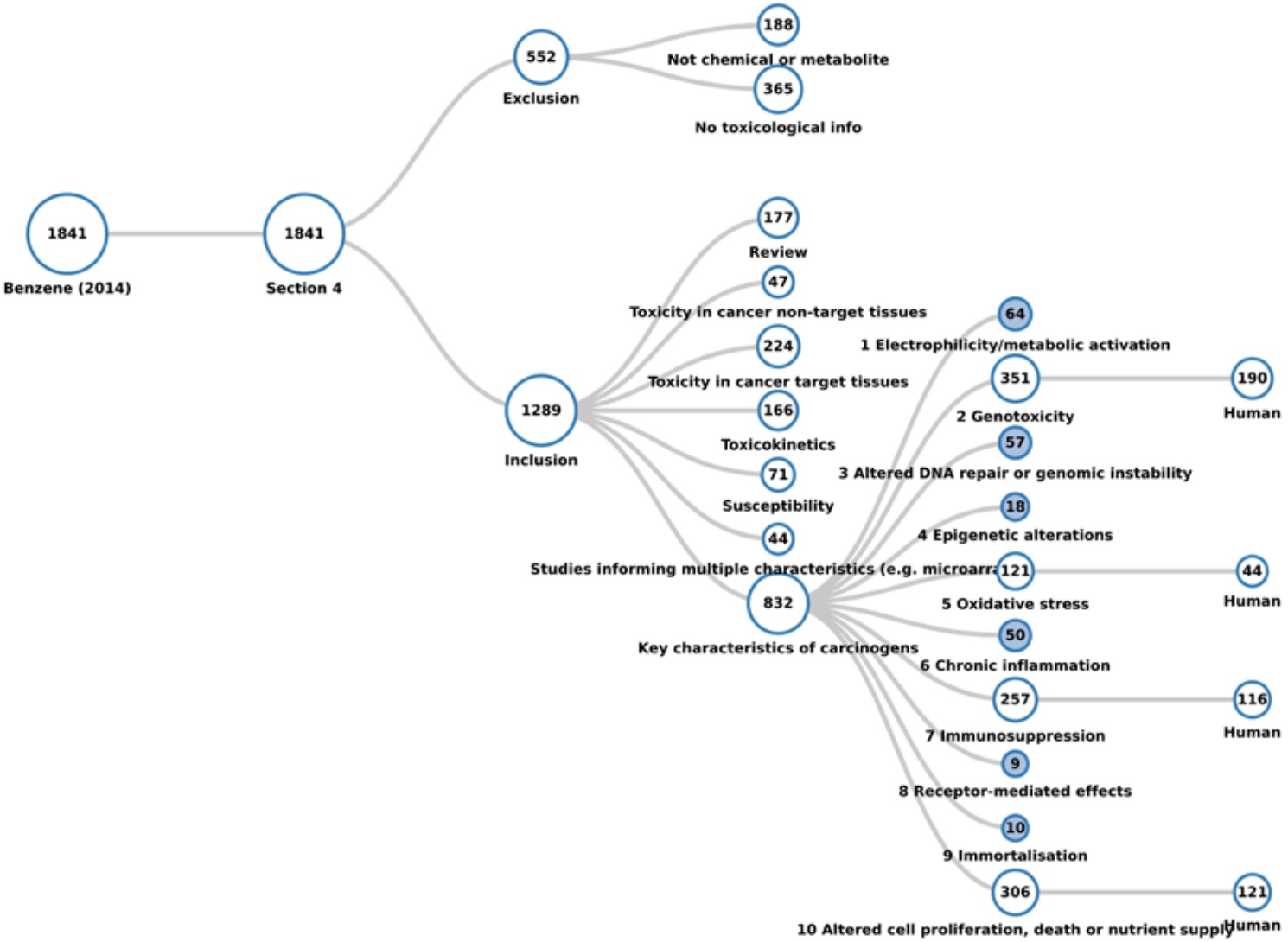
Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert P, Hecht SS, Bucher JR, Stewart BW, Baan R, Cogliano VJ and K Straif. *Env Health Persp.*, *in press*, http://ehp.niehs.nih.gov/15-09912/

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)

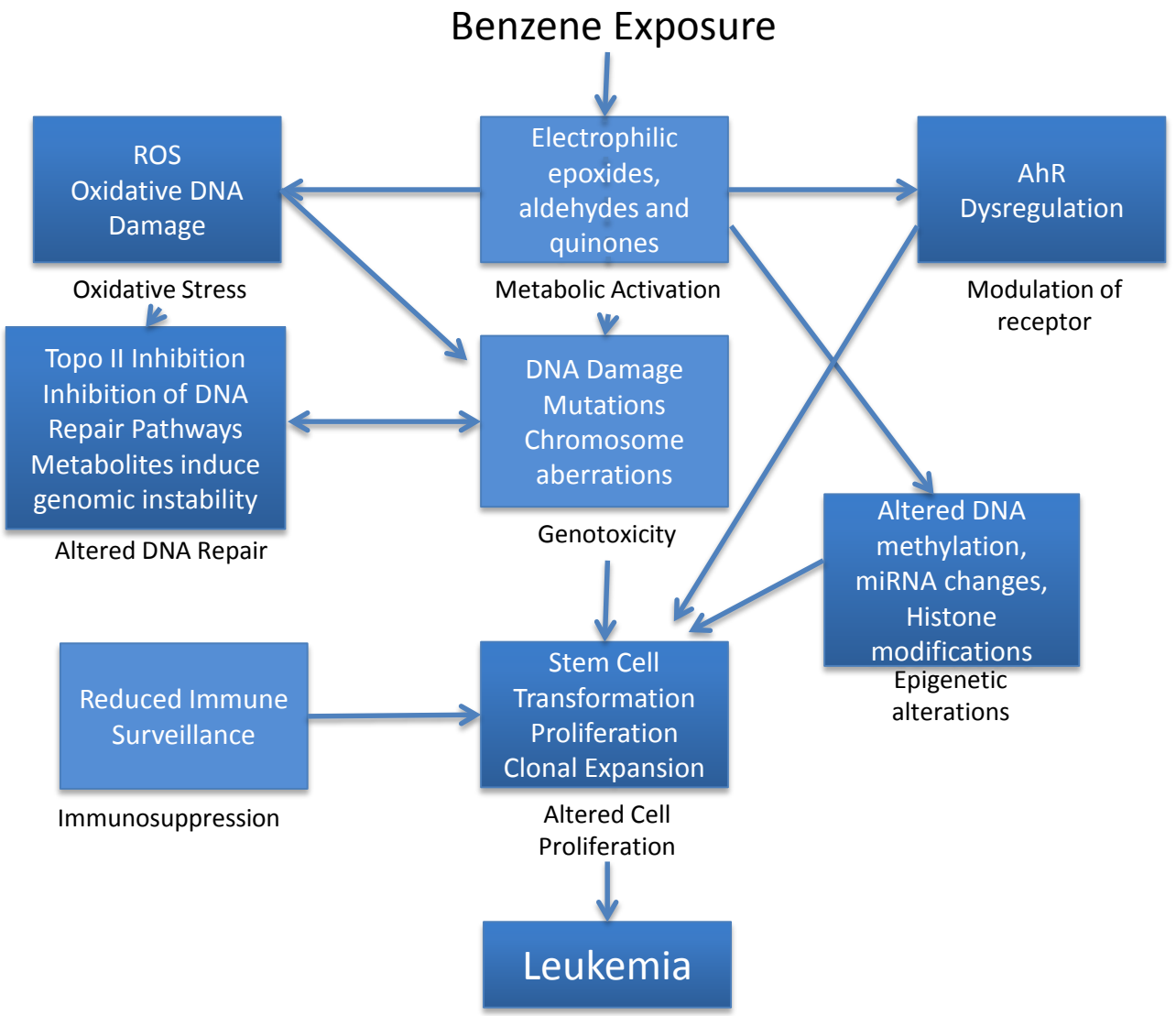
Characteristic	Examples of relevant evidence
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is Immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of exogenous 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

Benzene Mechanistic Data Search

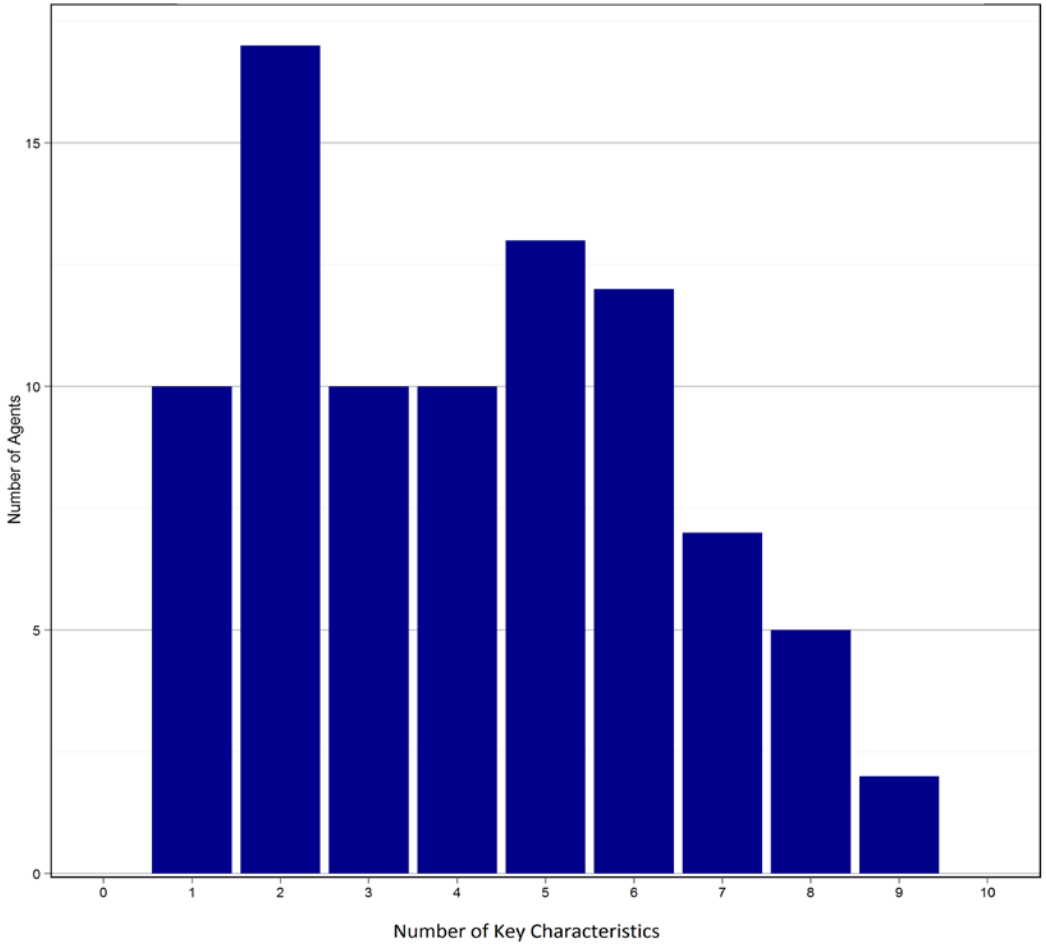
conducted using the Health Assessment Workplace Collaborative (HAWC) Literature Search tool (https://hawcproject.org/)



Benzene Example: An Adverse Outcome Network Involving 8 Key Characteristics



Number of IARC Group-1 Agents Demonstrating Multiple Key Characteristics



D. Krewski et al. in Monograph from IARC Working Group on 'Tumour-site Concordance and Mechanisms of Carcinogenesis', in press.

Implications of ‘key characteristics’

- Lays the groundwork for a structured evaluation of the strength of the mechanistic evidence base, and therefore its utility in supporting hazard classifications.
- Shows carcinogens tend to act through multiple mechanisms – separation into genotoxic and non-genotoxic actions of little value
- Allows development of credible Adverse Outcome Networks based on systematic review
- Could be developed for specific cancers and other adverse outcomes
- HT assays need to be developed based on characteristics and hallmarks. Current ones flawed.

An Agency-Academia Collaboration

- **IARC**: Kathryn Z. Guyton, Robert Baan and Kurt Straif
- **US EPA**: Catherine F. Gibbons, Jason M. Fritz, David M. DeMarini, Jane C. Caldwell, Robert Kavlock, Vincent Cogliano
- **NTP**: John R. Bucher
- **Academia**: Ivan Rusyn, Paul Lambert, Stephen S. Hecht, Bernard W. Stewart
- **Thun**: Christopher Portier
- **Other members** of the IARC WG: Lawrence Banks; Frederick A. Beland,; James A. Bond; Maarten C. Bosland; Bice Fubini; Bernard D. Goldstein; Kari Hemminki; Mark A. Hill; Charles Jameson; Agnes B. Kane; Daniel Krewski; Ronald Melnick; Jerry M. Rice; Leslie Stayner; Robert L. Ullrich; Harri Vainio; Paolo Vineis; Michael P. Waalkes; and, Lauren Zeise.
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