Biomarkers in Human Carcinogenesis

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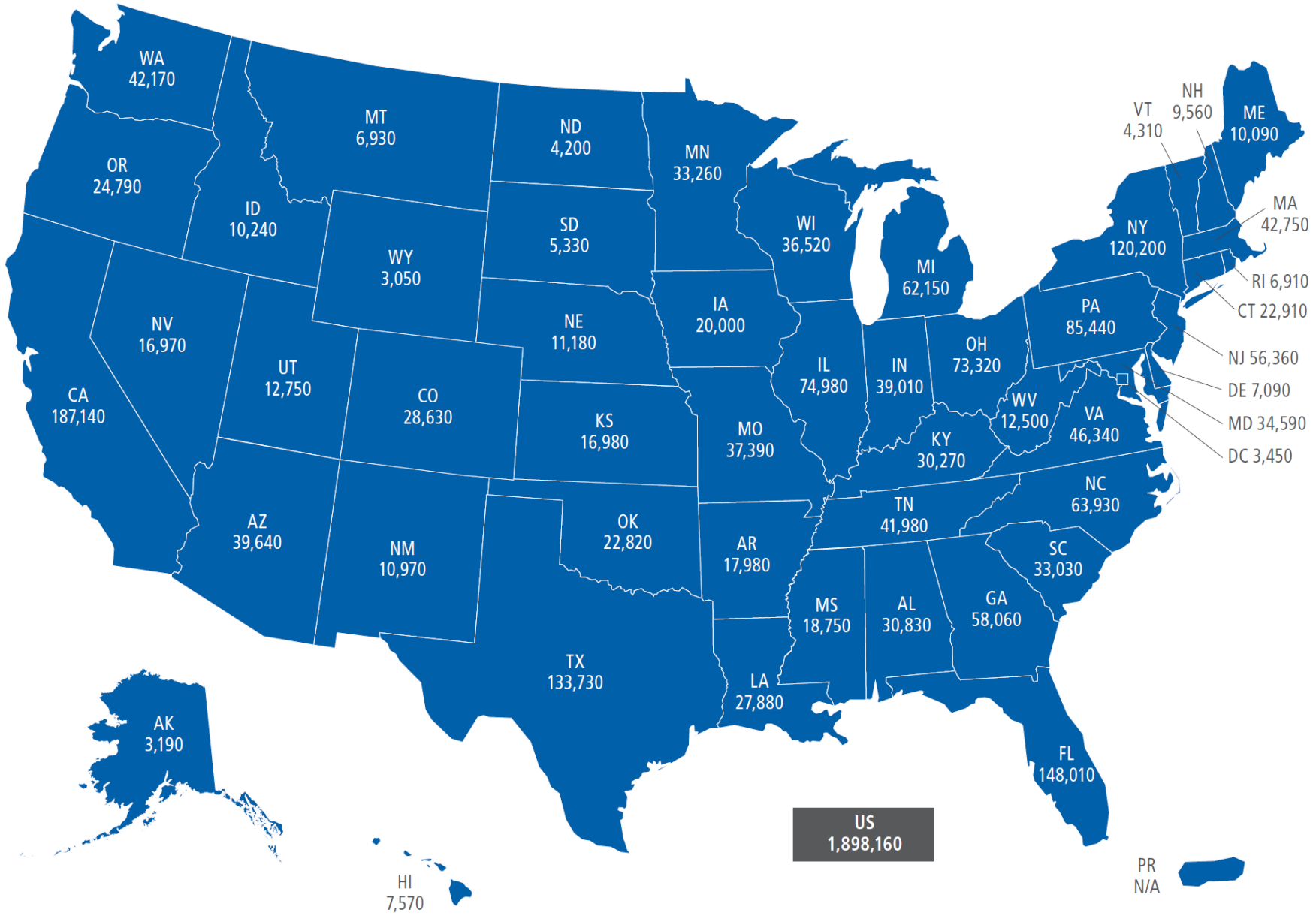
US Mortality, 2019 (Age-adjusted rates)*

Rank	Cause of death	No. of deaths	% of total deaths	Rate of mortality (per 10^5)
1	Heart Diseases	659,041	23.1	161.5
2	Cancer	599,601	21.0	146.2
3	Accidents (unintentional injuries)	173,040	6.1	49.3
4	Chronic lower respiratory diseases	156,979	5.5	38.2
5	Cerebrovascular diseases	150,005	5.3	37.0
6	Alzheimer disease	121,499	4.3	29.8
7	Diabetes mellitus	87,647	3.1	21.6
8	Nephritis (nephrotic syndrome)	51,565	1.8	12.7
9	Influenza and pneumonia	49,783	1.7	12.3
10	Intentional self-harm (Suicide)	47,511	1.7	13.9
	All other causes	758,167	26.6	

* Source, USCDC, 2019.

US New Cancer Cases estimated in 2021

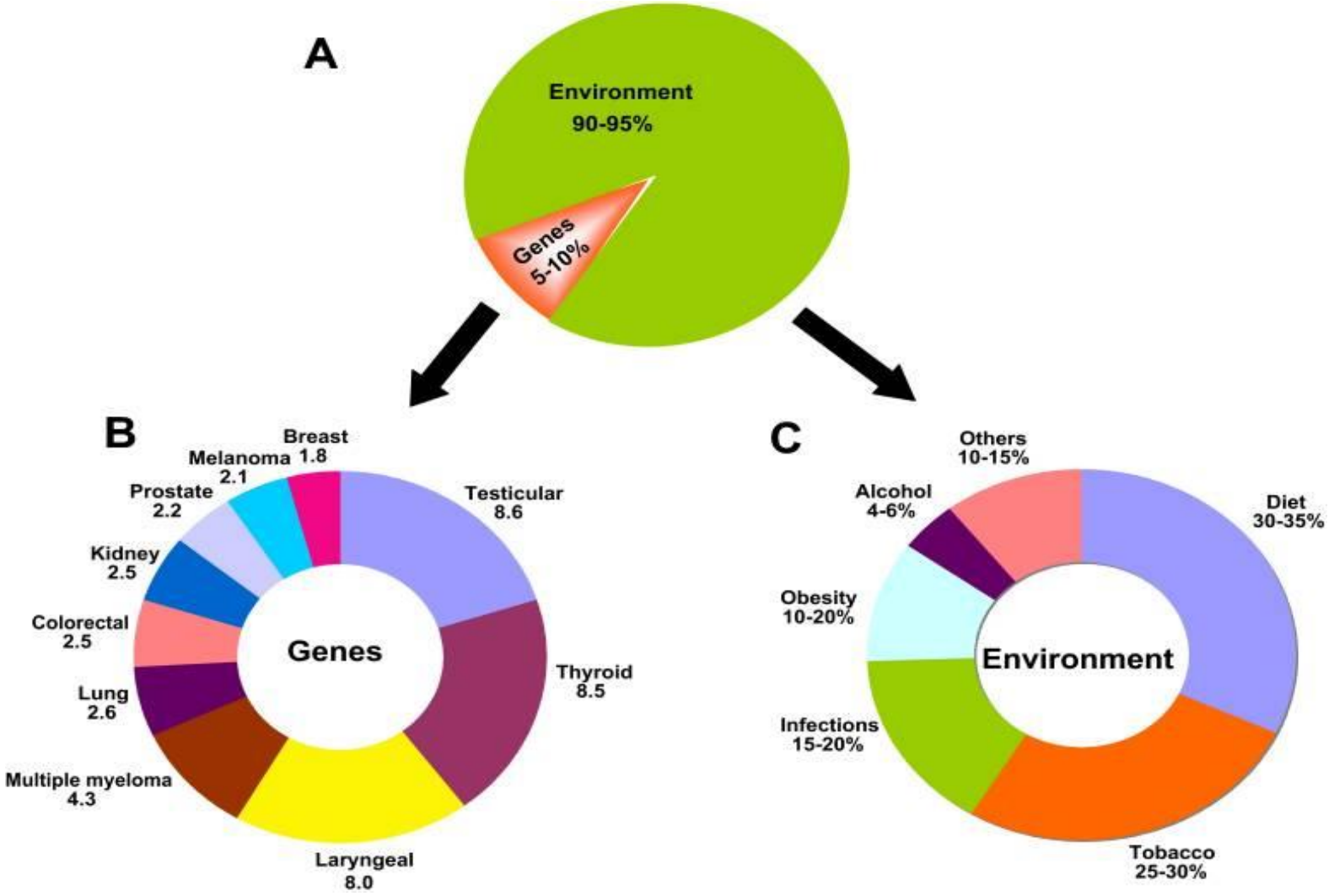


ACS, 2021

US Cancer Facts

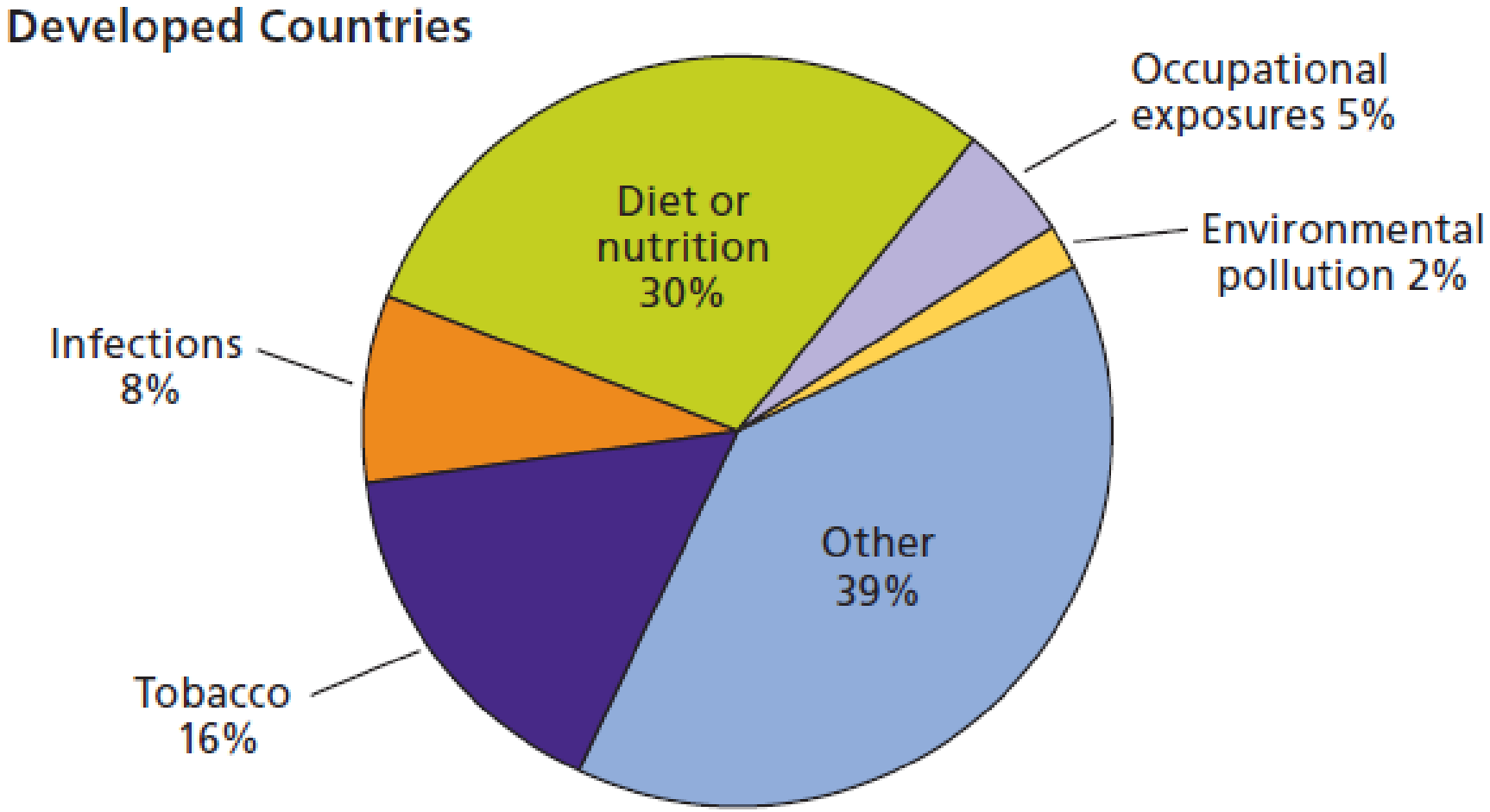
- More than 16.9 million Americans were alive with a history of cancer on January 1, 2019;
- About 1,898,160 new cancer cases are expected to be diagnosed in 2021;
- About 608,570 Americans are expected to die of cancer in 2021, about 1,670 deaths per day;
- At least 42%, about 750,000 new cases, are potentially avoidable (19% due to smoking and 18% due to a combination of excess body weight, physical inactivity, excess alcohol consumptions, and poor nutrition).

Causes of Cancer

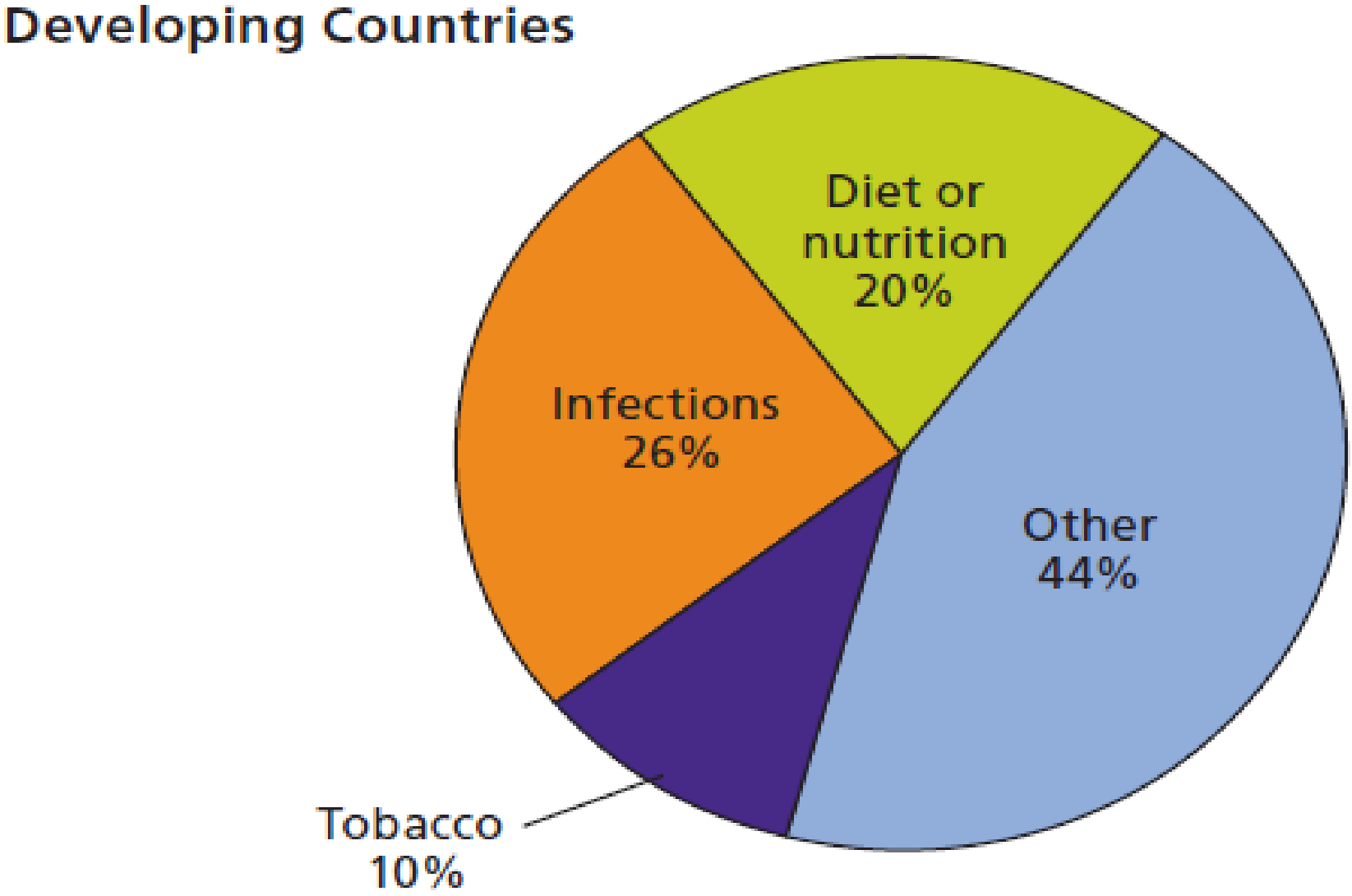


www.thelancet.com/oncology Published online May 9, 2012 DOI:10.1016/S1470-2045(12)70137-7

Worldwide Cancer Causes



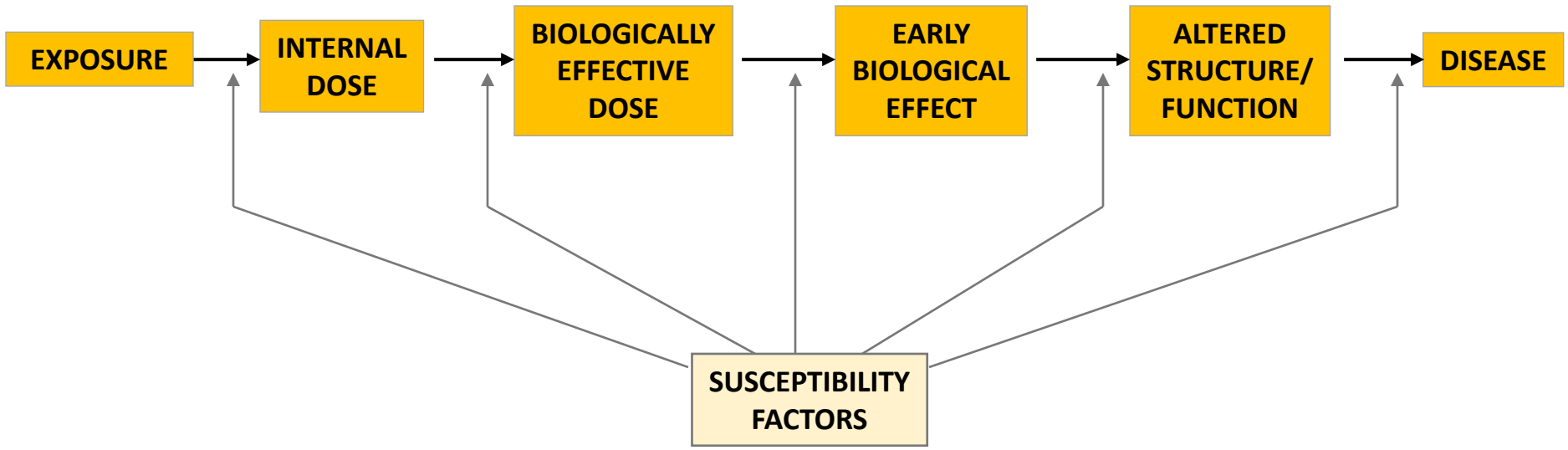
Worldwide Cancer Causes



Relative Importance of Various Cause of Cancer, United States

Risk factors	Estimated % of total cancer deaths attributable this factor
Tobacco	30
Adult diet/obesity	30
Sedentary lifestyle	5
Occupational factors	5
Family history of cancer	5
Virus/other biologic agents	5
Perinatal factors/growth	5
Reproductive factors	3
Alcohol	3
Socioeconomic status	3
Environmental pollution	2
Ionizing/ultraviolet radiation	2
Prescription drugs/medical procedures	1
Salt/other food additives and contaminants	1

The Biomarker Paradigm



NRC, 1987

Concepts

- A continuum of biological events, multiple stages, multiple factors;
- Indicators signaling these events or stages;
- Relationship between exposure and impairment;
- Modified by host-susceptibility factors at every stage.

Biomarkers

- Molecular, biochemical or cellular alterations that is measurable in biological media, such as tissues, cells, or fluids.

- Three types of biomarkers were proposed in 1987: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility.

Biomarkers of Exposure

- Indicate the presence and magnitude of the previous exposure to the chemical compounds;
- Measure chemical and its metabolite or its interaction with a biological molecule;
- Vary consistently and quantitatively with the extent of exposure;
- Specific for the environmental exposure of concern;
- Markers of internal dose usually are good biomarkers of exposure.

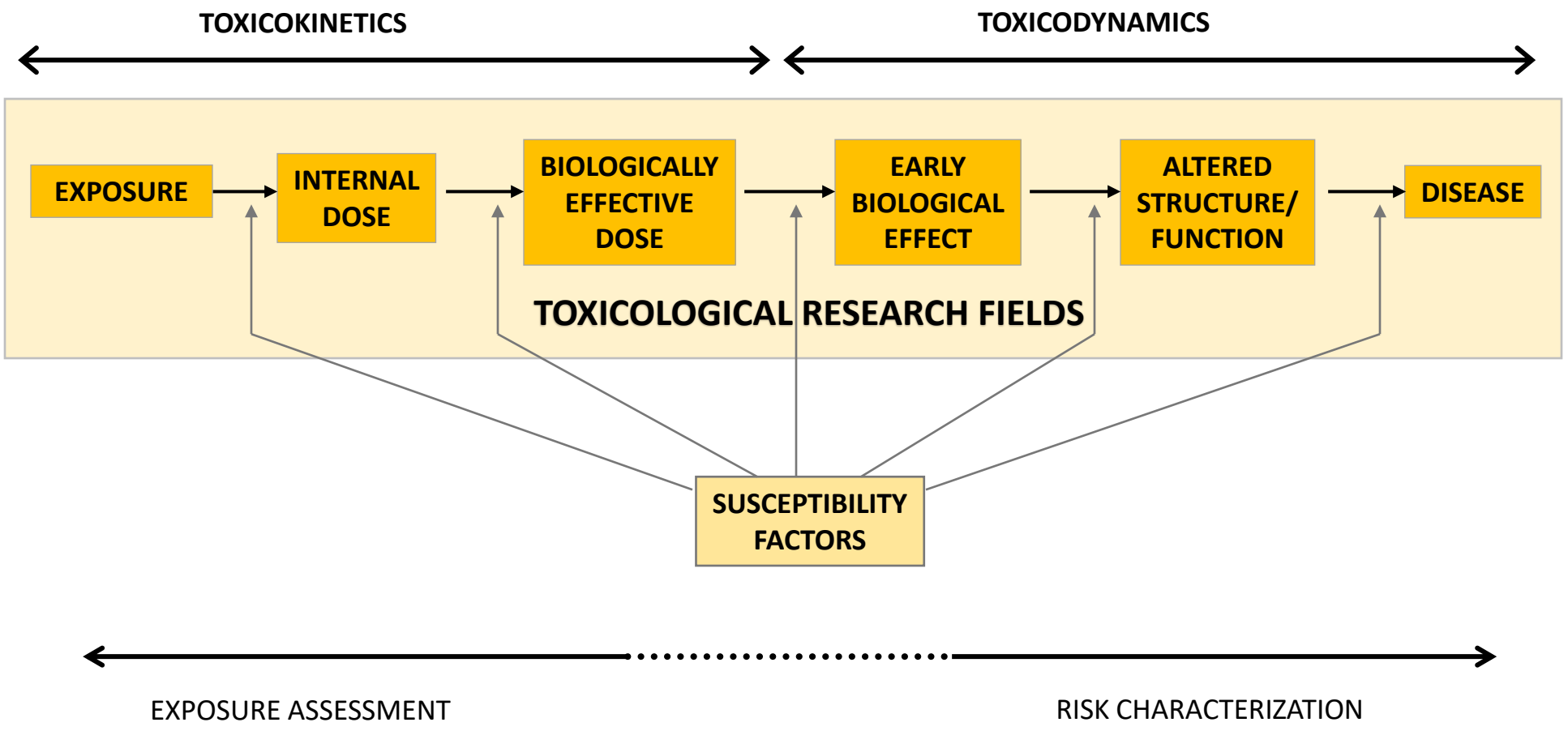
Biomarkers of Effect

- Indicate the presence and magnitude of biological response to exposure to the chemical compounds;
- A measurable biochemical, physiological, behavioral or other alteration within an organism that is associated with an established or possible health impairment or disease;
- Can be at levels of whole organism, organ function, tissue, individual cells and subcellular components.

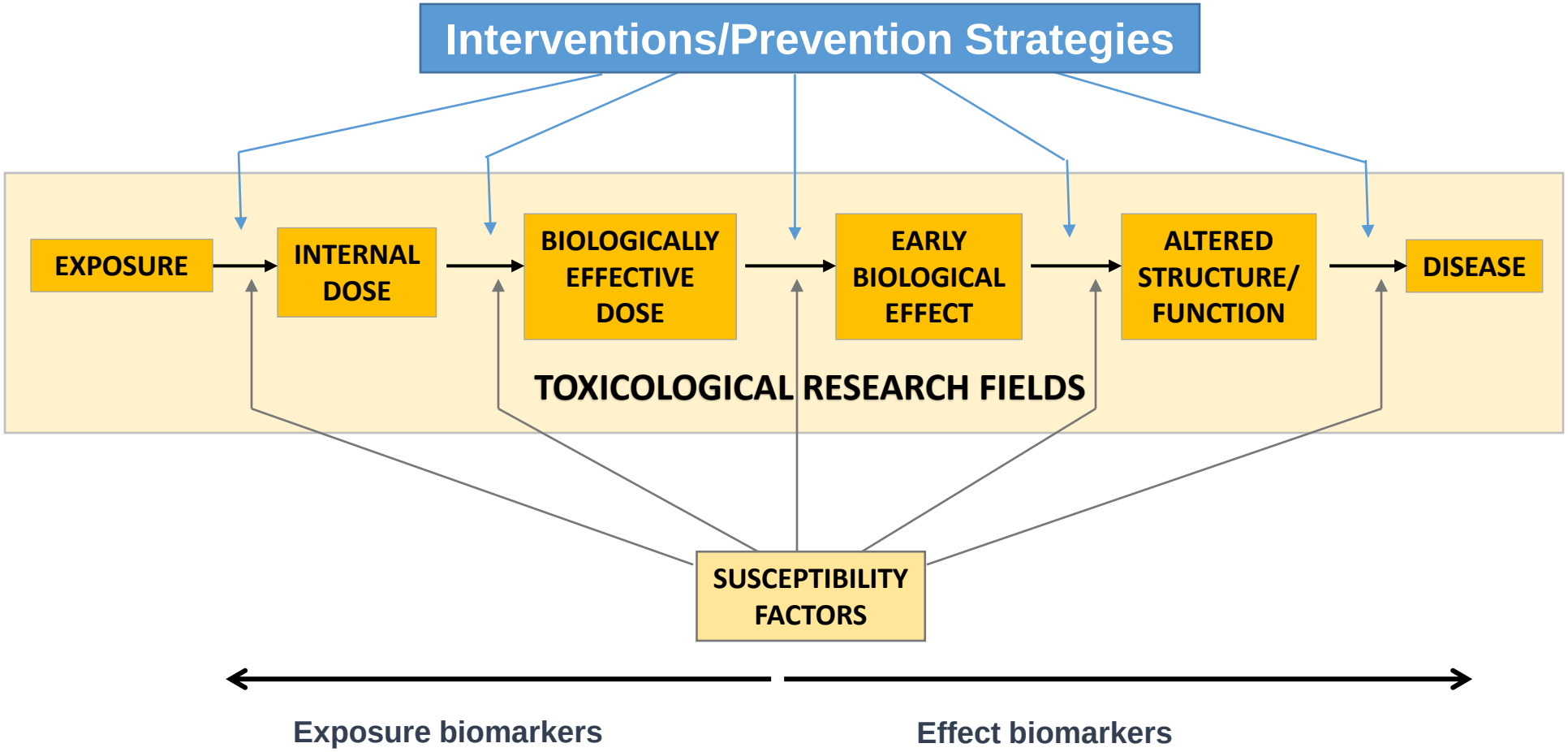
Biomarkers of Susceptibility

- An indicator or a measure of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical compound.
- Concerned with factors in kinetics and dynamics of uptake and metabolism of exogenous chemicals.
- The concept encompasses enzymes of activation and detoxification, repair enzymes, and changes in target molecules for toxic chemicals.

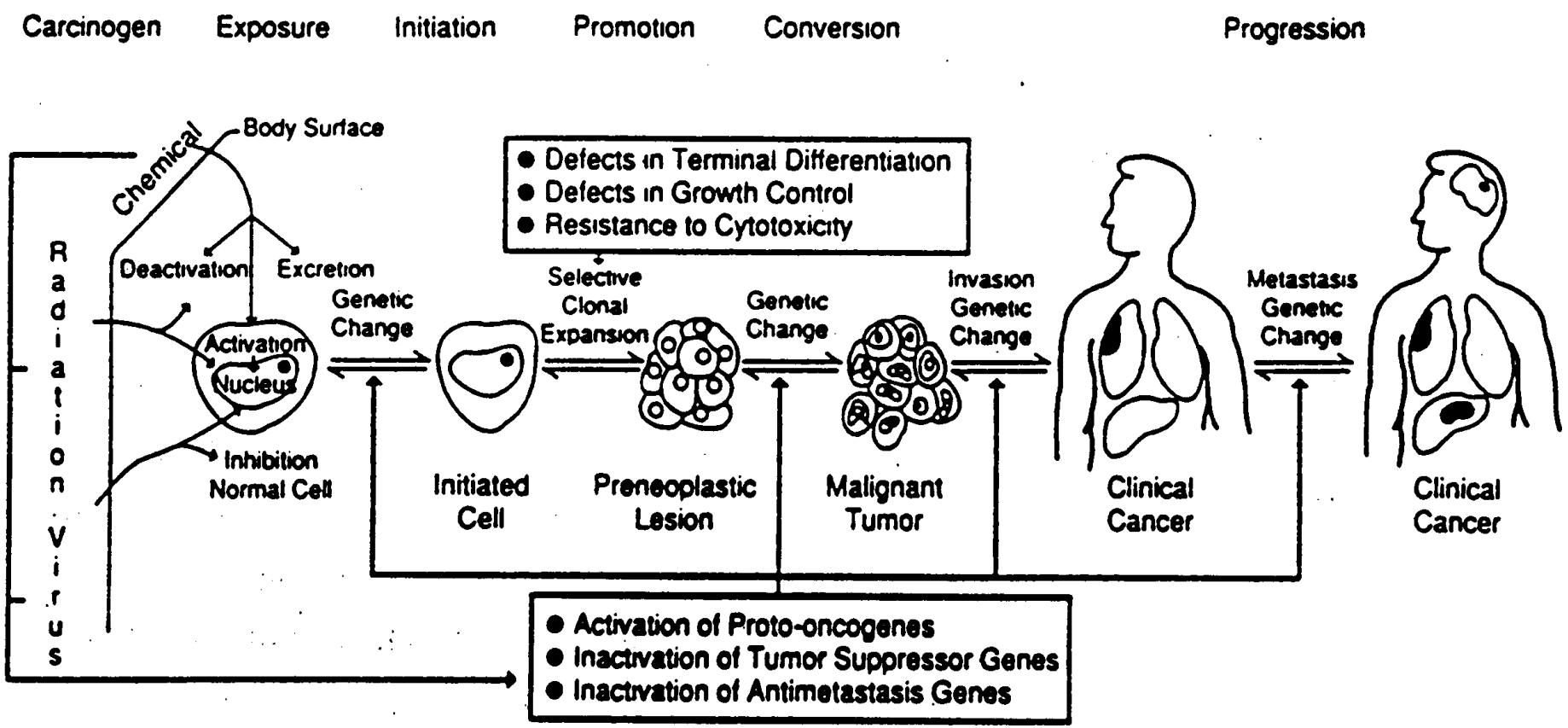
Biomarkers/Toxicology/Risk Assessment



Toxicology/Biomarkers/Prevention



Process of Carcinogenesis



Harris, 1995

Application of Biomarkers in Carcinogenesis

- To assess human carcinogen exposure for cancer etiology studies, via measurement of biomarkers of exposure, e.g., the internal dose of specific carcinogen;
- To assess carcinogenic effects at the early stage (initiation & promotion) with biomarkers of early effects;
- To assess carcinogenic effects at the middle and late stage (progression & metastasis) with biomarkers of later effects;
- To assess susceptibility in carcinogenic process with biomarkers of susceptibility;
- To assess efficacies of cancer prevention strategies with all three types of biomarkers.

Biomarkers of Early Effect

- Genotoxic Markers
  - DNA damage
  - Adducts: DNA adducts and Protein adducts
  - Oxidative damage
- Gene Mutation Markers
  - Somatic gene mutation in surrogate tissues
    - HPRT mutation
    - Glycophorin A
  - Gene mutation in target tissue
    - Oncogenes (Ras, Myc, etc.)
    - Tumor suppressor genes (Rb, APC, p53, etc.)

Biomarkers of Oxidative Damage

- 8-oxo-7,8-dihydroguanine (8-oxoGua)
- 8-oxo-7,8-dihydro-2’-deoxguanosine (8-oxodG) or 8-hydroxy-2’-deoxyguanosine (8-OHdG)
- Malondialdehyde (MDA)
- Nitrotyrosine
- F2-isoprostanes

Biomarkers of Mid-Effect

- Gene Regulation and Expression Markers
  - mRNA expression
  - micro RNAs and non-coding RNAs (Epigenetic markers)
  - Protein expression
- Cytogenetic Markers
  - Chromosomal aberrations
  - Sister chromatid exchanges
  - Micronuclei
- Enzymatic or Protein Markers
  - Organ-specific enzymes
  - Tumor markers (PSA)

Enzymatic Biomarkers

- Liver functions:
  - Alanine aminotransferase (ALT or SGPT)
    - Adults < 40 IU/L
  - Gamma Glutamyltransferase (GGT)
    - < 30 IU/L
  - Aspartate aminotransferase (AST, SGOT)
    - < 20 IU/L

Tissue	AST	ALT	Creatine Kinase
Heart	7800	450	473
Liver	7100	2850	<1
Skeletal muscle	5000	300	2500
Kidney	4500	1200	32
Pancreas	1400	130	<1
Spleen	700	80	<1
Lungs	500	45	<1

Biomarkers of Late-effect

- Structural and functional changes
  - Symptoms or Signs
- Early disease status
  - Polyps, dysplasia, etc.
- Tumor markers
- Advanced disease status
  - Metastasis markers

Tumor Markers

- A tumor marker is a substance found in an increased amount in the blood, other body fluids, or tissues that may suggest the presence of a type of cancer.

- Tumor markers are the biochemical or immunological counterparts of the differentiation state of the tumor, which represent the re-expression of substances produced normally by embryogenically related tissue.

- Tumor markers in dynamics may represent the tumor progression and can serve for the clinical diagnosis.

Tumor markers (examples)

- Alpha-fetoprotein (AFP, found in 1963)
  - A 70 kD glycoprotein, 4% carbohydrates
  - < 15 ng/mL
- Carcinoembryonic antigen (CEA, found in 1965)
  - A family of glycoproteins, 50% carbohydrates
  - < 5.0 ng/mL
- Prostate specific antigen (PSA, found in 1980)
  - A 28.43 kD glycoprotein, 7% carbohydrates
  - < 4.0 ng/mL

Tumor markers (examples)

- Carbohydrate-related tumor markers
  - Antigens in tumor surface
  - CA 15-3 for Breast/ovarian cancers
  - CA 125 for Ovarian/endometrial cancers
  - CA 549 for breast/ovarian cancers
  - CA 19-5 for GI and pancreatic cancers
  - CA 19-9 for pancreatic and GI cancers

Biomarkers of Susceptibility (Examples)

- Phenotypic expression markers
  - Enzyme activities
  - Phase 1 and 2 metabolites
  - Protein (enzymes) expressions
- Genetic polymorphisms markers
  - Single nucleotide changes
  - Homozygous vs. heterozygous
  - Deletions
  - Insertions

Genes and Genetic Variations

- Human genome
  - 3 billion base pairs
  - 30,000 genes
  - 100,000 proteins
  - Variations in expression of proteins
- Single nucleotide polymorphisms (SNPs)
  - 10 million SNPs in the genome or 1/ 300 base pairs
  - Minimal effect in creating susceptibility to disease
  - Many genetic variants to have relatively weak contributions to chronic diseases, such as cancer, diabetes
  - Interactions with environment and non-genetic factors play important role in susceptibility.

Polymorphisms of CYP450s

- CYP1A1:
  - MspI and Ile (AUU/AUC/AUA)-Val (GUU/GUC/GUA) variants (A-G),
  - MspI/MspI or Val/Val genotype have a high risk of possessing a mutant p53,
  - associated with lung and breast cancers.
- CYP1B1:
  - Leu (CUU/CUC/CUA)-Val in codon 432,
  - associated with breast and prostate cancers.
- CYP1A2:
  - No genotypic polymorphism has been found;
  - Phenotypic variations;
  - Associated with colorectal and bladder cancers.
  - Urinary caffeine metabolites are good biomarkers for evaluation of polymorphic phenotypes of CYP1A2 and NAT2.

Polymorphisms of CYP450s

- CYP2D6:
  - Also called debrisoquine hydrolase;
  - Poor metabolizers (PMs), lower risks for lung and bladder cancer;
  - Extensive metabolizers (EMs), high risks?
- CYP2E1:
  - Three types of genetic polymorphism;
  - DraI, RsaII, and PstI;
  - C1/C1, C1/C2, and C2/C2.

Polymorphisms of CYP450s

- CYP2C9:
  - Polymorphic was found in metabolizing tolbutamide, an antidiabetic drug;
  - Three polymorphic types were identified, called *1, *2, and *3.
- CYP2C19
  - Polymorphic was found in metabolizing mephenytoin, an anticonvulsant drug;
  - Three polymorphic types were also identified, called m1, m2, and m3.
- PMs and EMs were found for both CYP450s;
- Only 2-5% Caucasians for PMs, however, 13-23% Orientals for PMs.

Ethnic variations of genetic polymorphisms of metabolic enzymes

Polymorphism	Caucasian (%)	Asian (%)	African-American (%)
CYP1A1-MspI	7-14	33	23
CYP1A1-Ile-Val	3	20	2
CYP1A1-MspI (intron 7)	<1	<1	9
CYP2D6*	7-10	1	NA
CYP2E1-DraI	9	31	9
CYP2E1-PstI	2	24	5
CYP2E1-RsaI	2	27	2
mEH-113 H/H	8	NA	NA
mEH-139R/R	5	NA	NA
GSTM1**	43-52	48-60	22-35
GSTT1**	15-20	60	22
NAT2***	40-70	32-60	68
Paraoxonase	50	Variable	Variable

* Poor metabolizer alleles; **null (deleted) alleles; ***slow acetylator alleles.

Variations in DNA Repair

- DNA repair systems are responsible for maintaining the integrity of the genome by minimizing replication errors, removing DNA damage, and minimizing deleterious rearrangements arising via aberrant recombination;

- There are many studies focused on identification of single nucleotide polymorphisms (SNPs) in DNA repair genes;

- A great deal of substitution variants have been found in genes in DNA repair pathways, which lead to variations in critical DNA repair enzymes.

Measurement of Global Repair Capacity

- Endogenous cellular repair capacities are more than adequate to compensate for background DNA damage (global repair capacity);
- Massive or frequent exposure to exogenous genotoxic agents may saturate the DNA repair capacity leading to human genetic disease;
- Global repair capacity assay can test this balance.

Major Polymorphic DNA Repair Genes

- ERCC
- hOGG
- RAD51
- XPC
- XPD
- XRCC
- BRAC1 and BRAC2

Genetic variations in DNA repair pathways

Pathway	No. of genes in pathway*	No. of genes Screened	No. of variants identified	No. of alleles >2% frequency
Base excision repair	25	19	68	15
Nucleotide excision repair	33	16	51	17
Double strand break repair	~30	11	31	8
Mismatch repair	7	6	41	13
Damage recognition & cell cycle checkpoint	~30	6	13	4
Total**	>110	51	177	50

*Genes with roles in more than pathways are counted in all relevant pathways. **The total is unique genes and variants, not the sum of the column.

Summary

- Biomarker concept, once was proposed, has been widely accepted by toxicological and many biomedical research fields;
- Biomarkers can be divided into biomarkers of exposure, effects, and susceptibility, and can be classified as genotypic and phenotypic biomarkers based on the current measurement tools;
- Biomarkers in carcinogenesis can be classified as early-, middle- and late-effect markers based on the specific events or stages in carcinogenic process;
- Biomarkers have been applied to assess human carcinogen exposure for cancer etiology studies; to assess carcinogenic effects at the early stages (initiation & promotion) and the middle and late stages(progression & metastasis);
- Biomarkers have been widely used to assess efficacies of cancer prevention strategies, and biomarkers of susceptibility were extensively used to study variations in cancer rates and individual/ethnic cancer risks.