Molecular biomarkers of effect in the causality determination process: an important piece of the puzzle?

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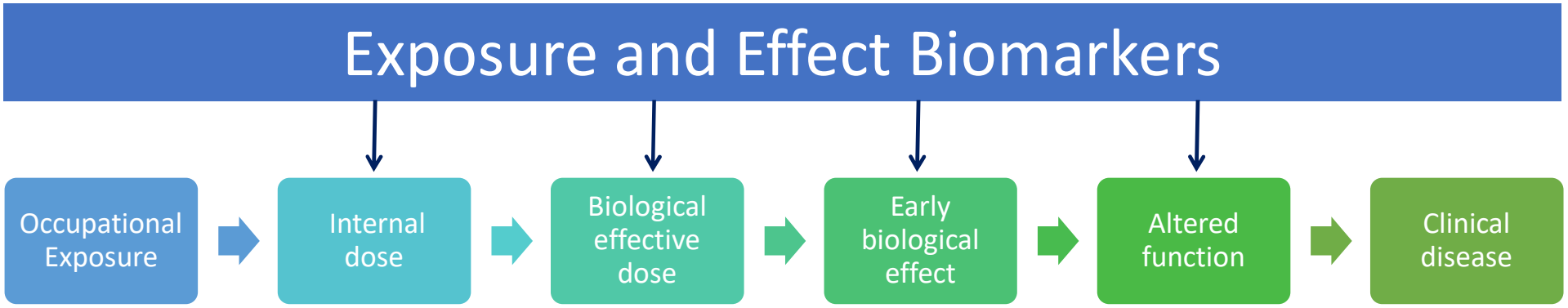
Graphical abstract of my talk

«Biomarkers of effect»

Biomarkers of effect are the **quantifiable** changes that an individual endures, which **indicates an exposure** to a compound and may indicate a resulting **health effect**.



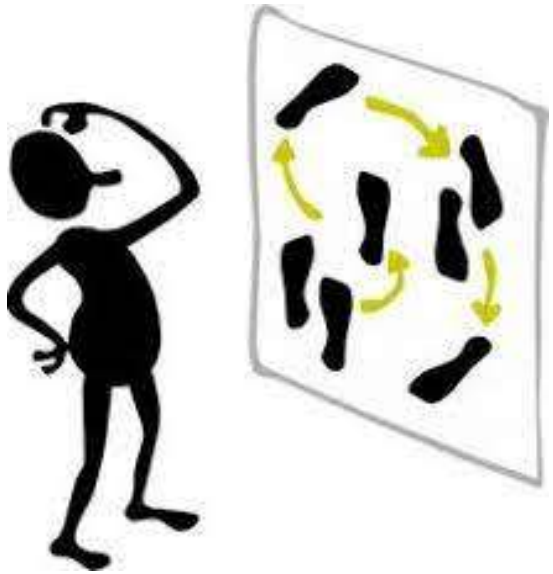
«Causality determination» in occupational health

An **injury or illness** is considered by Occupational Safety and Health Administration to be "**work related**" if an event or exposure in the work environment either **caused or contributed** to the resulting condition or significantly aggravated a pre-existing condition.

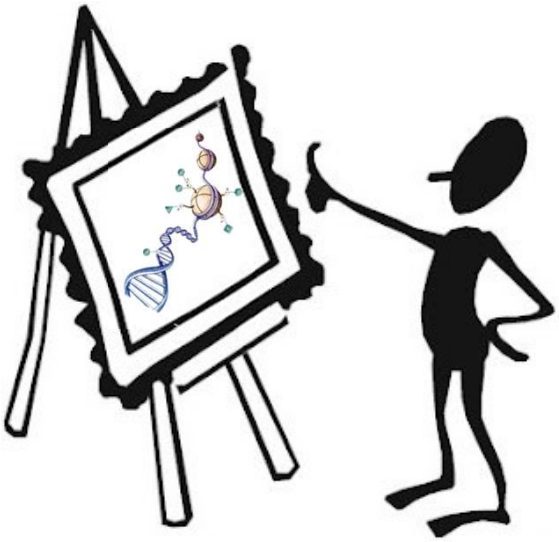
https://www.osha.gov

The challenge

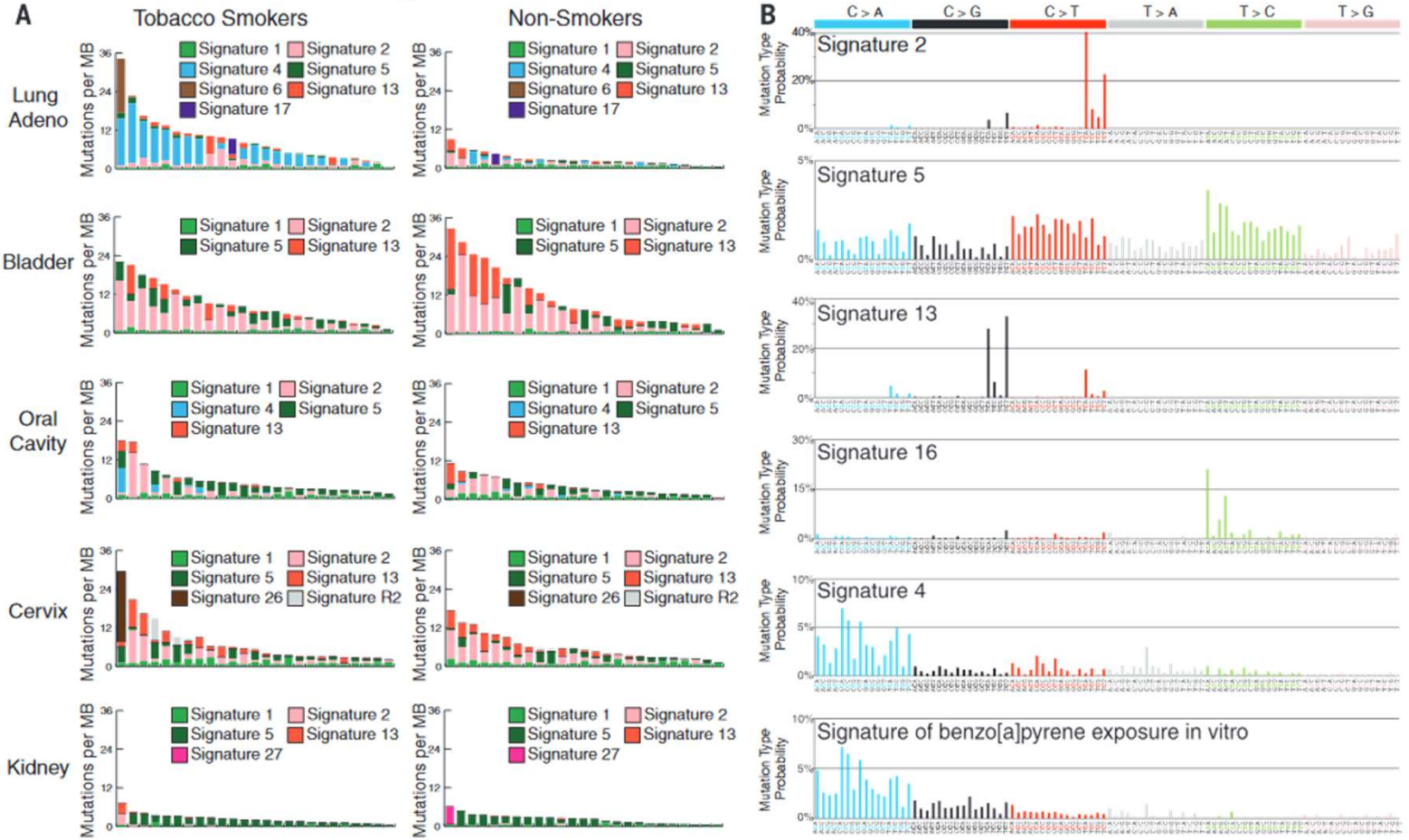
- Establishing a work-related cause of a disease is one of the most challenging aspects of occupational medicine.
- ALL diseases are multicausal
- Occupational diseases can be:
  - mainly caused by factors at work (e.g. mesothelioma)
  - diseases in which work plays a minor role in causation (e.g. back pain).



Environmental events can be permanently registered by our cells



Mutational signatures associated with tobacco smoking



Tobacco smoking increases the risk of at least 17 classes of cancer

Genome analyses of cancer tissues in:
-2,490 tobacco smokers
-1,063 never smokers

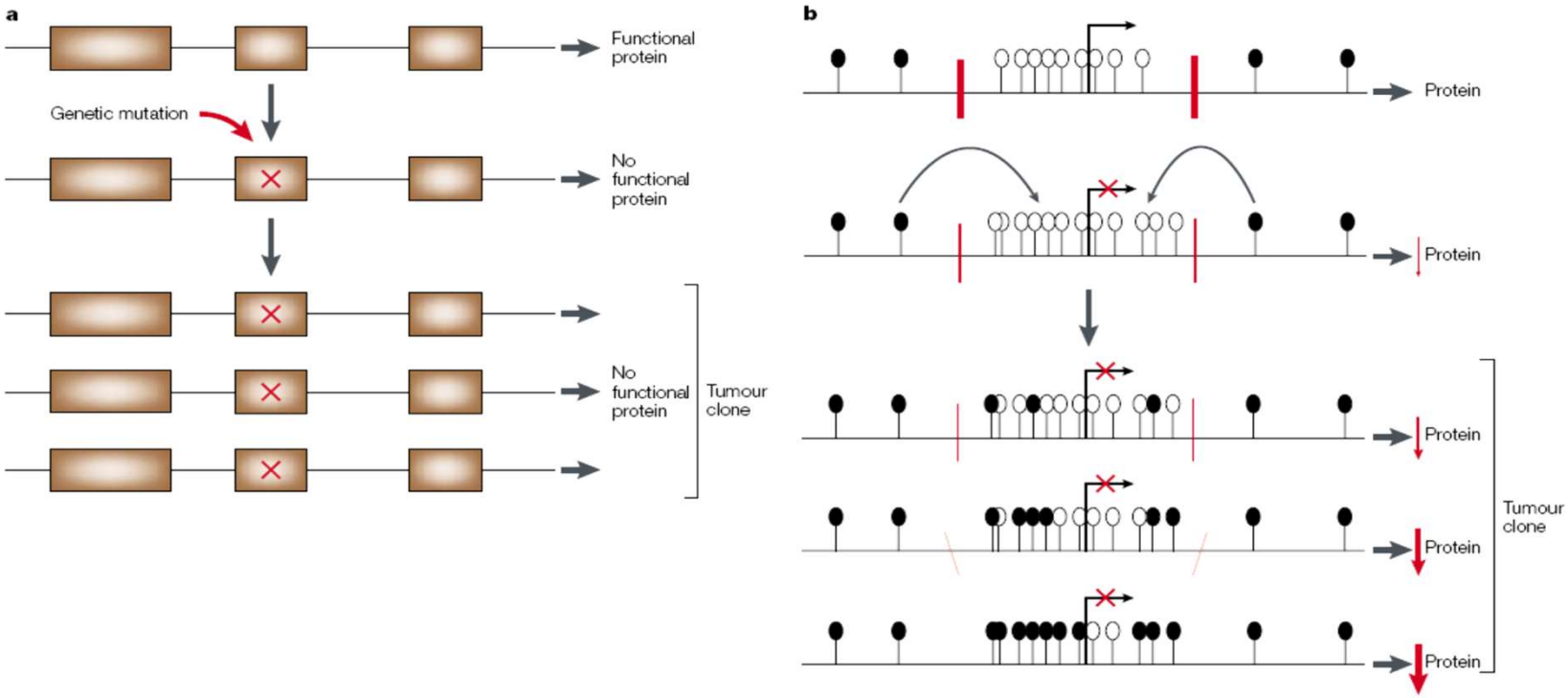
Alexandrov LB, et al. Mutational signatures associated with tobacco smoking in human cancer. Science. 2016 Nov;354(6312):618-622.

PROs and CONs

- Mutational signature is specific.
- Exposure signature was identified in cancer tissue, in the fully developed disease.
- Mutational signature is not dynamic by definition.

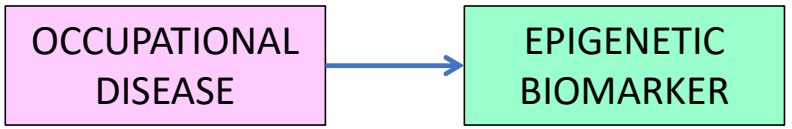
EPIGENETICS

DNA mutations versus “epimutations”

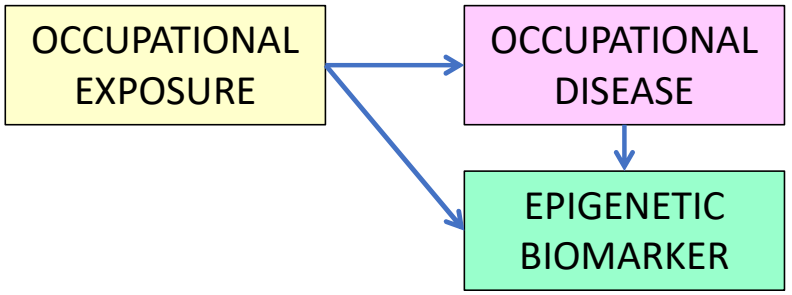


Jones & Baylin Nature Rev 3:415, 2002

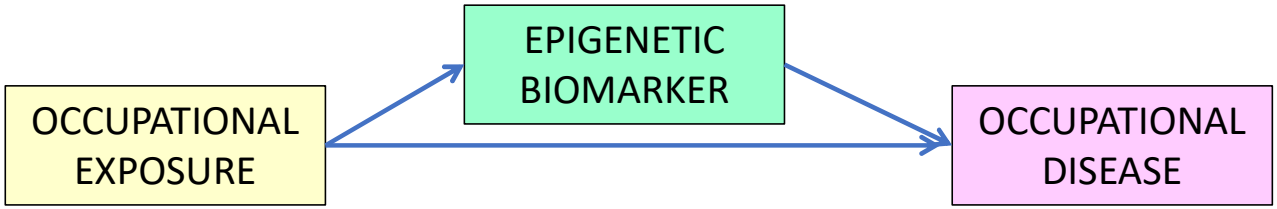
The possible scenarios



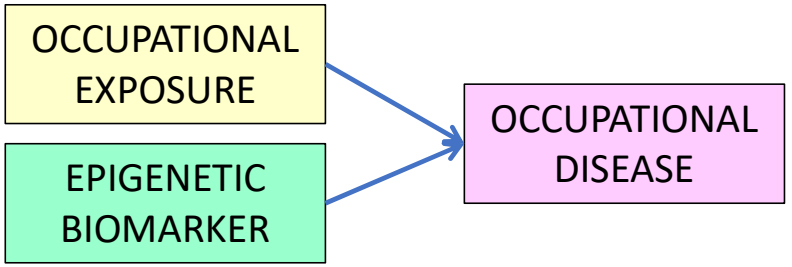
REVERSE CAUSATION



CONFOUNDED



CAUSALITY and MEDIATION



INDEPENDENT and both CAUSAL

An example

BENZENE EXPOSURE → ACUTE MYELOID LEUKEMIA

?

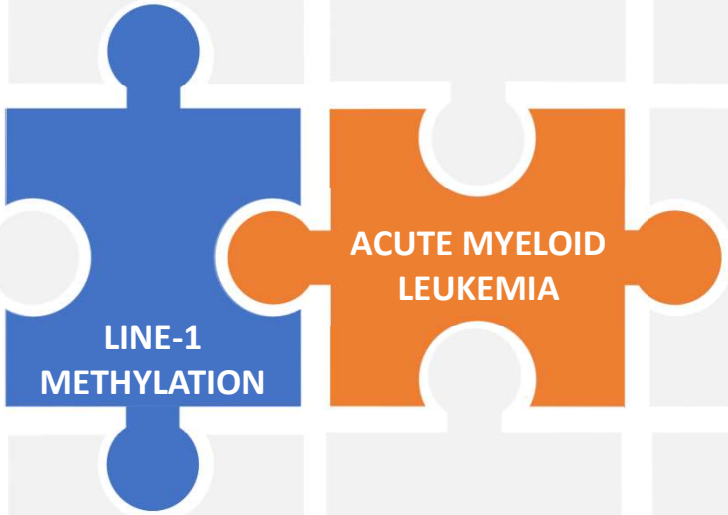
DNA METHYLATION

Benzene and Acute Myeloid Leukemia

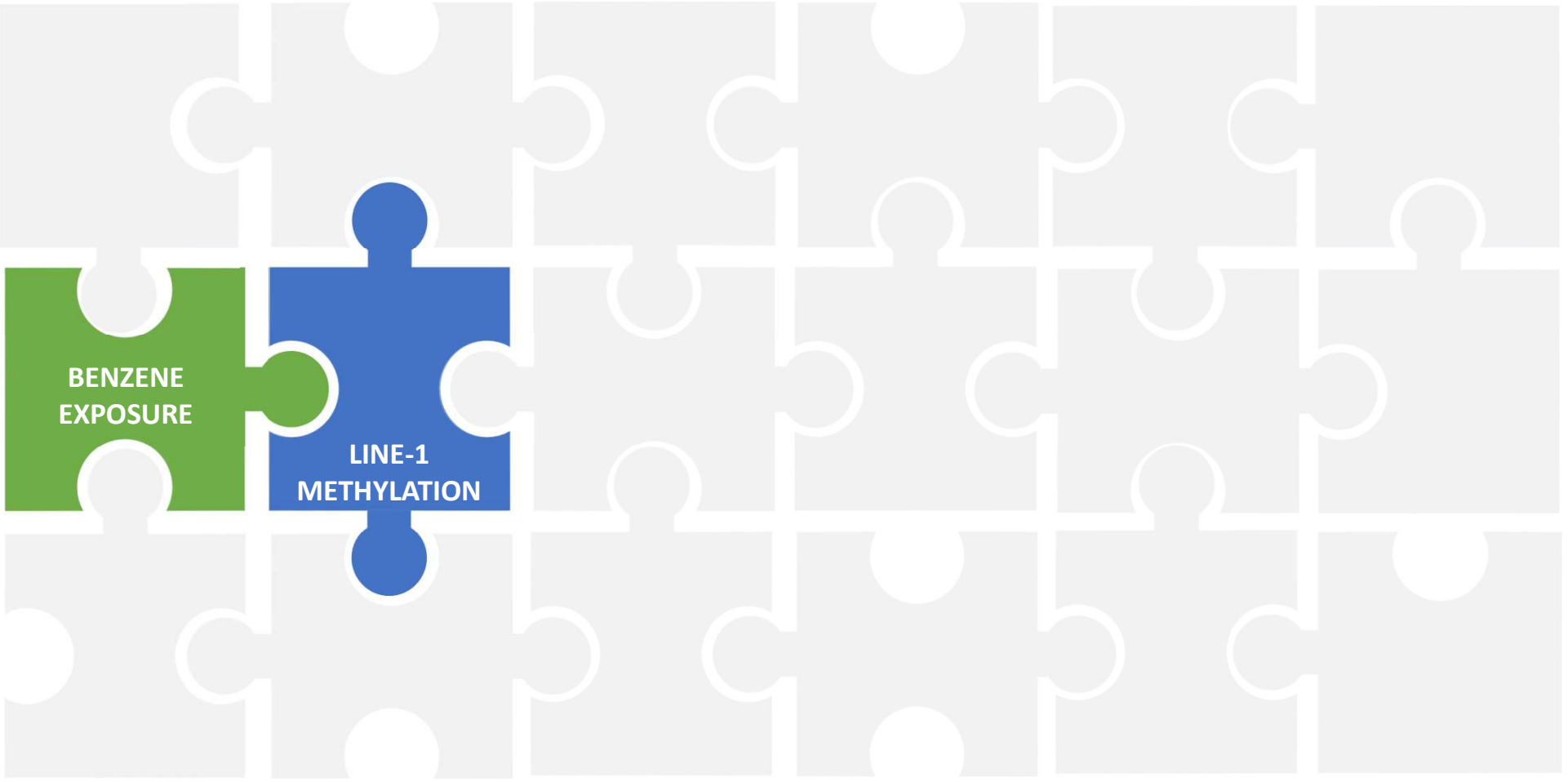
- Benzene (C_6H_6) is the smallest and most stable aromatic hydrocarbon
- Benzene is an airborne pollutant of industrial and general environment
- Benzene is a well known human leukemogen (group 1 IARC) (IARC, 1987)
- Benzene exposure in human populations is causally related to the development of AML (Savitz and Andrews, 1997).
- An increased risk of leukemia was reported in different occupational settings characterized by high exposure, but conflicting results for low-exposures

DNA methylation and Acute myeloid leukemia

- Leukemia cells exhibit an abnormal methylation pattern.
- The CpG islands in the promoters of some genes become hypermethylated.
- The global genome of cancer cells undergoes hypomethylation, up to 20-60% less than normal cells.
- **LINE-1 methylation** is highly correlated with global methylation, thus undergoes hypomethylation in cancer cells.



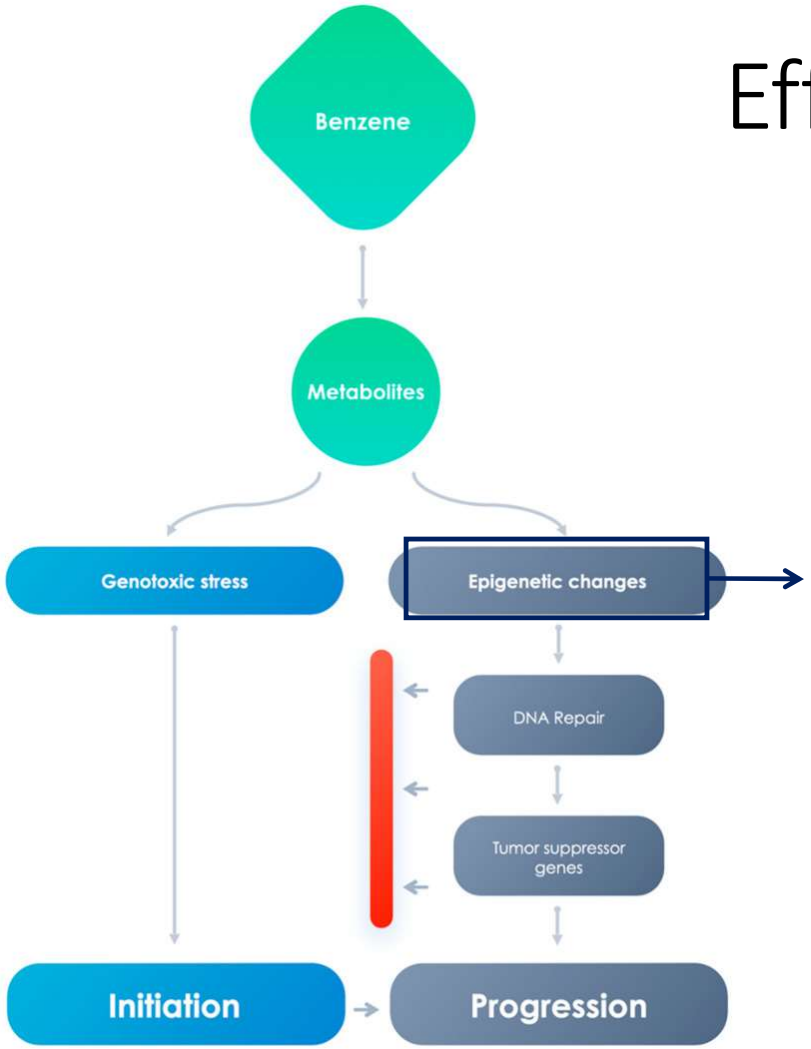
WEBINAR Settling a debate about theoretical bases of occupational disease

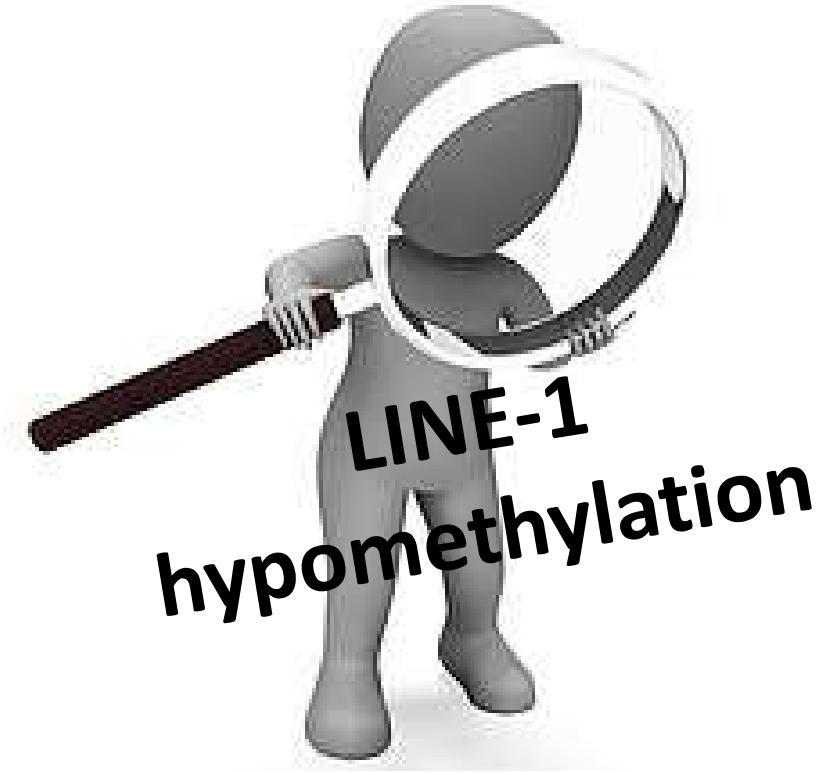


SOCIETÀ ITALIANA DI MEDICINA DEL LAVORO
Fondata nel 1929
SIML

WEBINAR Settling a debate about theoretical bases of occupational disease

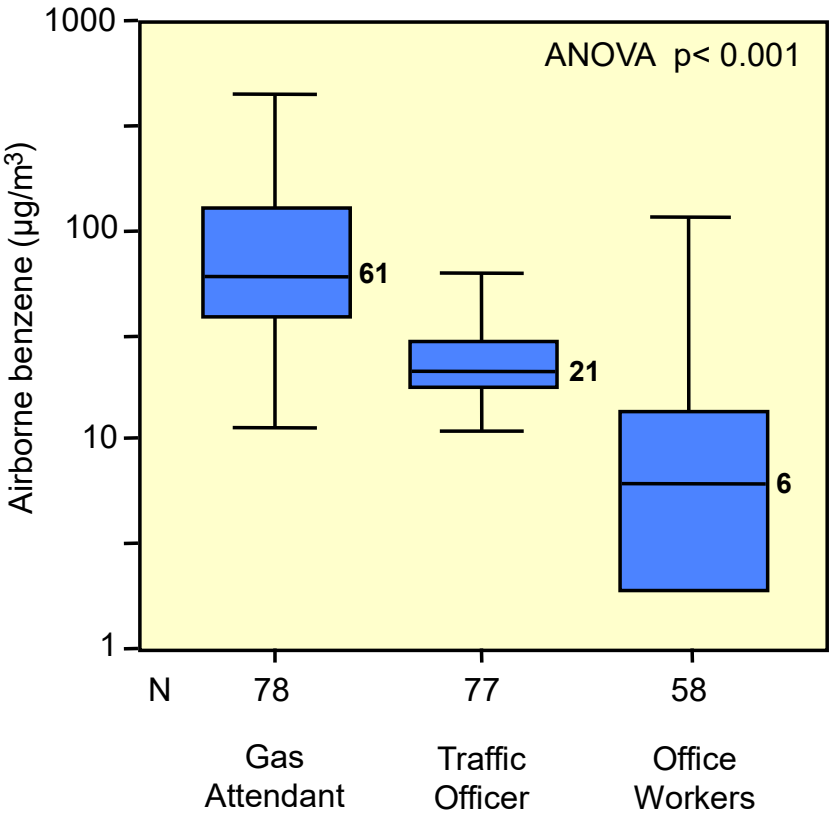
Effects of benzene exposure

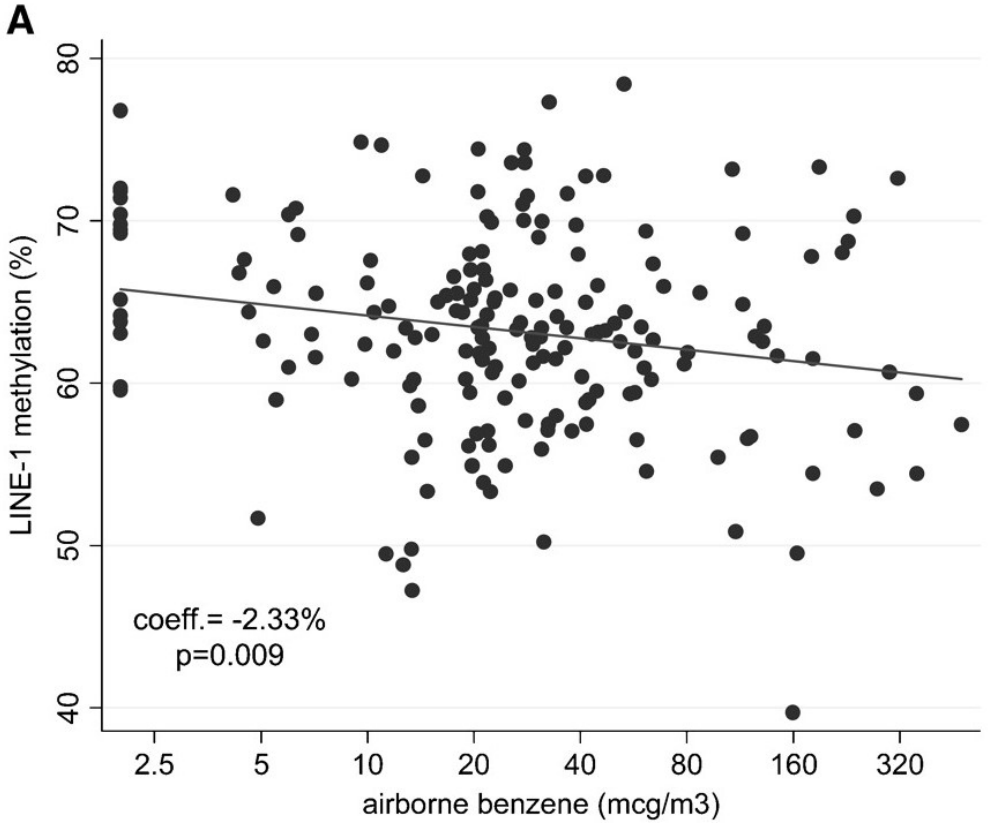




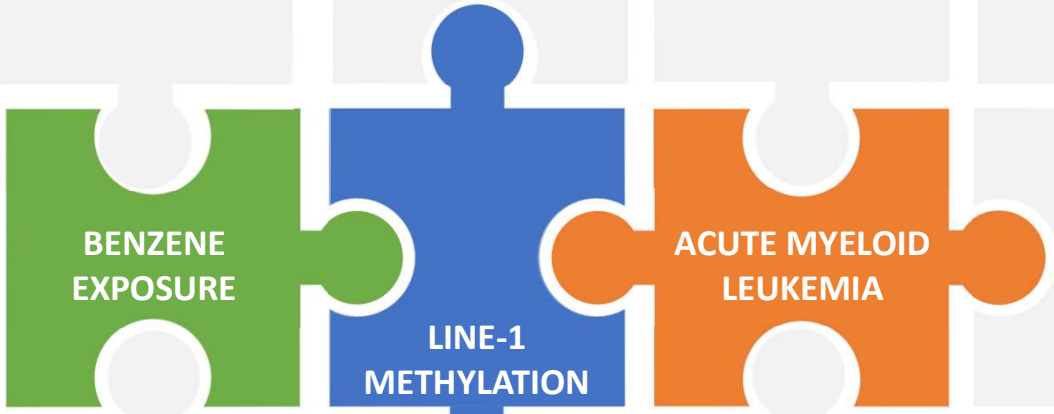
Spatari G, et al. Cancers (Basel). 2021;13(10):2392.

Benzene exposure and LINE-1 methylation





Bollati et al. Cancer Res 2007;67:876-880



SOCIETÀ ITALIANA DI MEDICINA DEL LAVORO
Fondata nel 1929
SIML

WEBINAR **Settling a debate about theoretical bases of occupational disease**

HOWEVER…

Not only AML…

Guarrera *et al. Clinical Epigenetics* (2015) 7:133
DOI 10.1186/s13148-015-0164-3

Clinical Epigenetics

RESEARCH Open Access

Gene-specific DNA methylation profiles and LINE-1 hypomethylation are associated with myocardial infarction risk

Simonetta Guarrera[1,2†], Giovanni Fiorito[1,2†], N. Charlotte Onland-Moret[3], Alessia Russo[1,2], Claudia Agnoli[4], Alessandra Allione[1,2], Cornelia Di Gaetano[1,2], Amalia Mattiello[5], Fulvio Ricceri[6], Paolo Chiodini[7], Silvia Polidoro[1], Graziella Frasca[8], Monique W. M. Verschuren[3,9], Jolanda M. A. Boer[9], Licia Iacoviello[10], Yvonne T. van der Schouw[3], Rosario Tumino[8], Paolo Vineis[1,11], Vittorio Krogh[4], Salvatore Panico[5], Carlotta Sacerdote[6] and Giuseppe Matullo[1,2*]

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PLOS ONE

LINE-1 Hypomethylation in Blood and Tissue Samples as an Epigenetic Marker for Cancer Risk: A Systematic Review and Meta-Analysis

Martina Barchitta[1], Annalisa Quattrocchi[1], Andrea Maugeri[1], Manlio Vinciguerra[2,3]*, Antonella Agodi[1]*

1 Department GF Ingrassia, University of Catania, Catania, Italy, **2** University College London, Institute for Liver and Digestive Health, Royal Free Campus, London, United Kingdom, **3** Gastroenterology Unit, Department of Medical Sciences, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 05 October 2021
doi: 10.3389/fped.2021.685310

Analysis of Global and Local DNA Methylation Patterns in Blood Samples of Patients With Autism Spectrum Disorder

María Victoria García-Ortiz[1,2,3†], María José de la Torre-Aguilar[1,4†], Teresa Morales-Ruiz[1,2,3], Antonio Gómez-Fernández[1,4], Katherine Flores-Rojas[1,5,6], Mercedes Gil-Campos[1,5,6], Pilar Martín-Borreguero[1,7], Rafael R. Ariza[1,2,3], Teresa Roldán-Arjona[1,2,3] and Juan Luis Pérez-Navero[1,4,8]*

scientific reports

Check for updates

OPEN

Aging-associated distinctive DNA methylation changes of LINE-1 retrotransposons in pure cell-free DNA from human blood

Wardah Mahmood[1,9], Lars Erichsen[1,9], Pauline Ott[1], Wolfgang A. Schulz[2], Johannes C. Fischer[3], Marcos J. Arauzo-Bravo[4,5], Marcelo L. Bendhack[6], Mohamed Hassan[7,8] & Simeon Santourlidis[1]✉

WEBINAR Settling a debate about theoretical bases of occupational disease

Not only benzene…

Rapid DNA Methylation Changes after Exposure to Traffic Particles

Andrea Baccarelli[1,2], Robert O. Wright[2,3], Valentina Bollati[1], Letizia Tarantini[1], Augusto A. Litonjua[3], Helen H. Suh[2], Antonella Zanobetti[2], David Sparrow[4], Pantel S. Vokonas[4], and Joel Schwartz[2]

[1]Laboratory of Environmental Epigenetics, Department of Environmental and Occupational Health, University of Milan & IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy; [2]Department of Environmental Health, Harvard School of Public Health; [3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School; and [4]VA Normative Aging Study, Veterans Affairs Boston Healthcare System and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts

Received: 12 December 2020 | Revised: 11 June 2021 | Accepted: 14 July 2021

DOI: 10.1002/em.22451

RESEARCH ARTICLE

Environmental and Molecular Mutagenesis | Environmental Mutagenesis and Genomics Society | WILEY

Global and gene-specific promoter methylation, and micronuclei induction in lead-exposed workers: A cross-sectional study

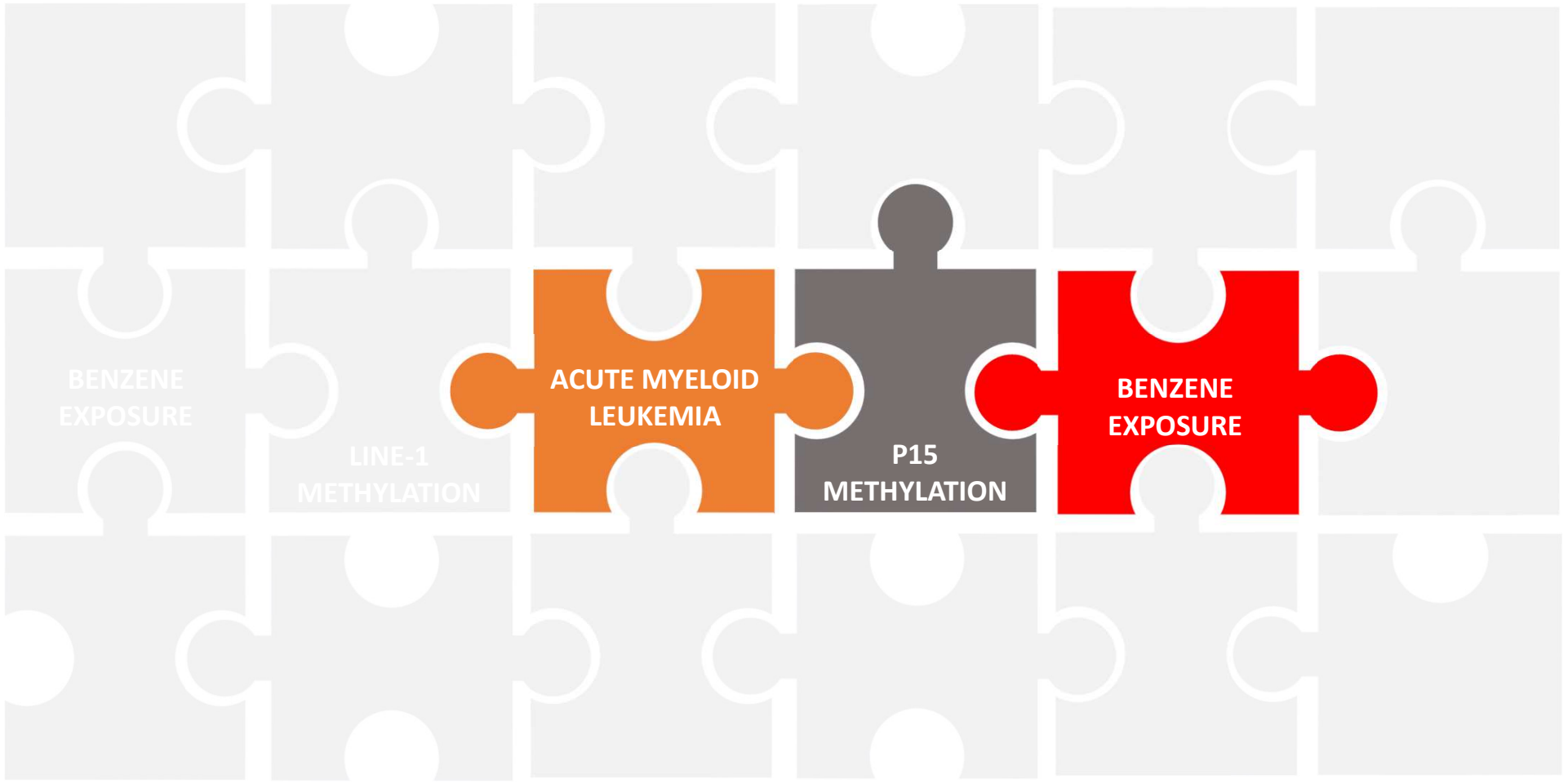
Kan Wang[1,2] | Yu Meng[1] | Tuanwei Wang[1] | Yuting Tu[1] | Shiyang Gong[1] | Guanghui Zhang[3] | William Au[4] | Zhaolin Xia[1]

Mediterranean Diet and Particulate Matter Exposure Are Associated With LINE-1 Methylation: Results From a Cross-Sectional Study in Women

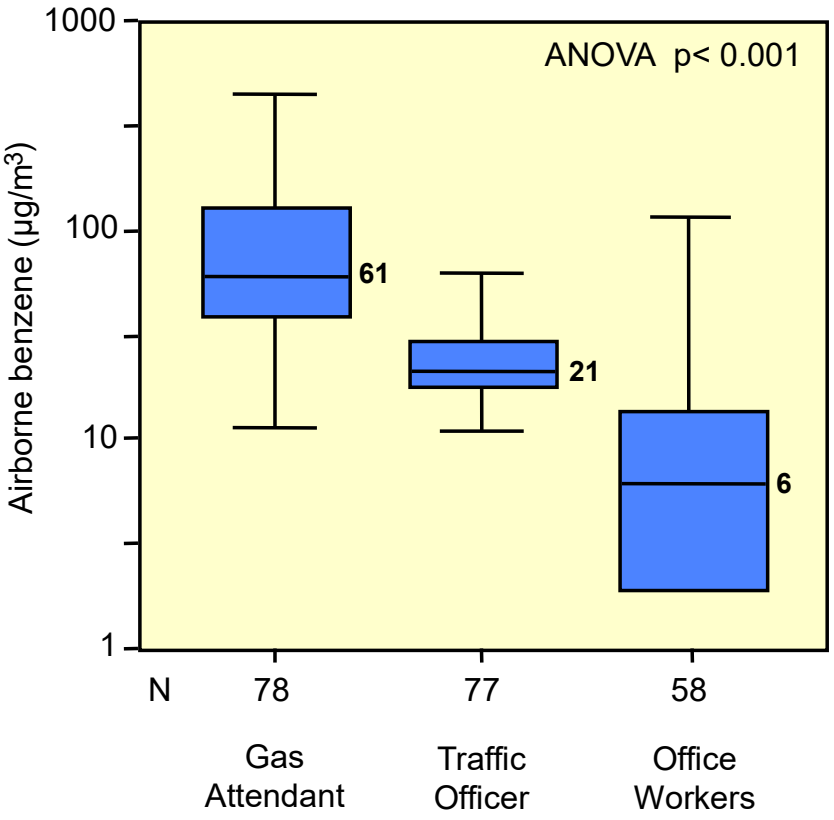
Martina Barchitta[1], Andrea Maugeri[1], Annalisa Quattrocchi[1], Germana Barone[2], Paolo Mazzoleni[2], Alfio Catalfo[3], Guido De Guidi[3,4], Maria Giovanna Iemmolo[5], Nunzio Crimi[5] and Antonella Agodi[1*]

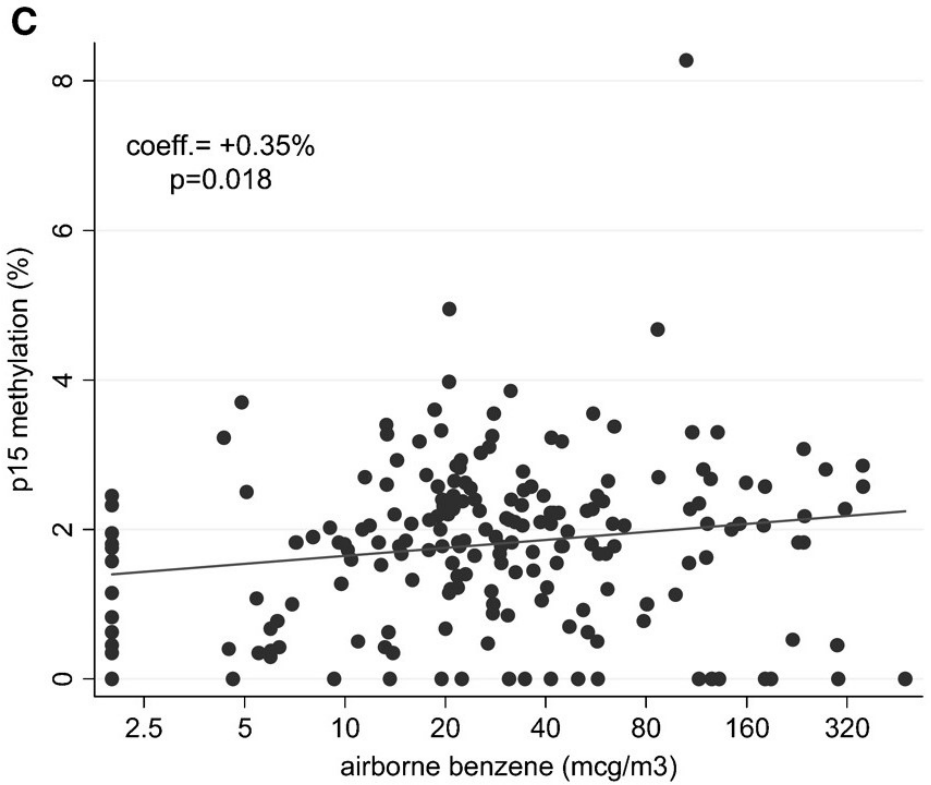
[1]Department of Medical and Surgical Sciences and Advanced Technologies "GF Ingrassia", University of Catania, Catania, Italy
[2]Department of Biological, Geological and Environmental Sciences, University of Catania, Catania, Italy
[3]Department of Chemical Science, Section of Photochemistry and Photobiology, University of Catania, Catania, Italy
[4]Research Centre for the Analysis, the Monitoring and Methodology for Environmental Risk Assessment, University of Catania, Catania, Italy
[5]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

WEBINAR Settling a debate about theoretical bases of occupational disease


BENZENE EXPOSURE
LINE-1 METHYLATION
ACUTE MYELOID LEUKEMIA
P15 METHYLATION
BENZENE EXPOSURE

Benzene exposure and p15 methylation





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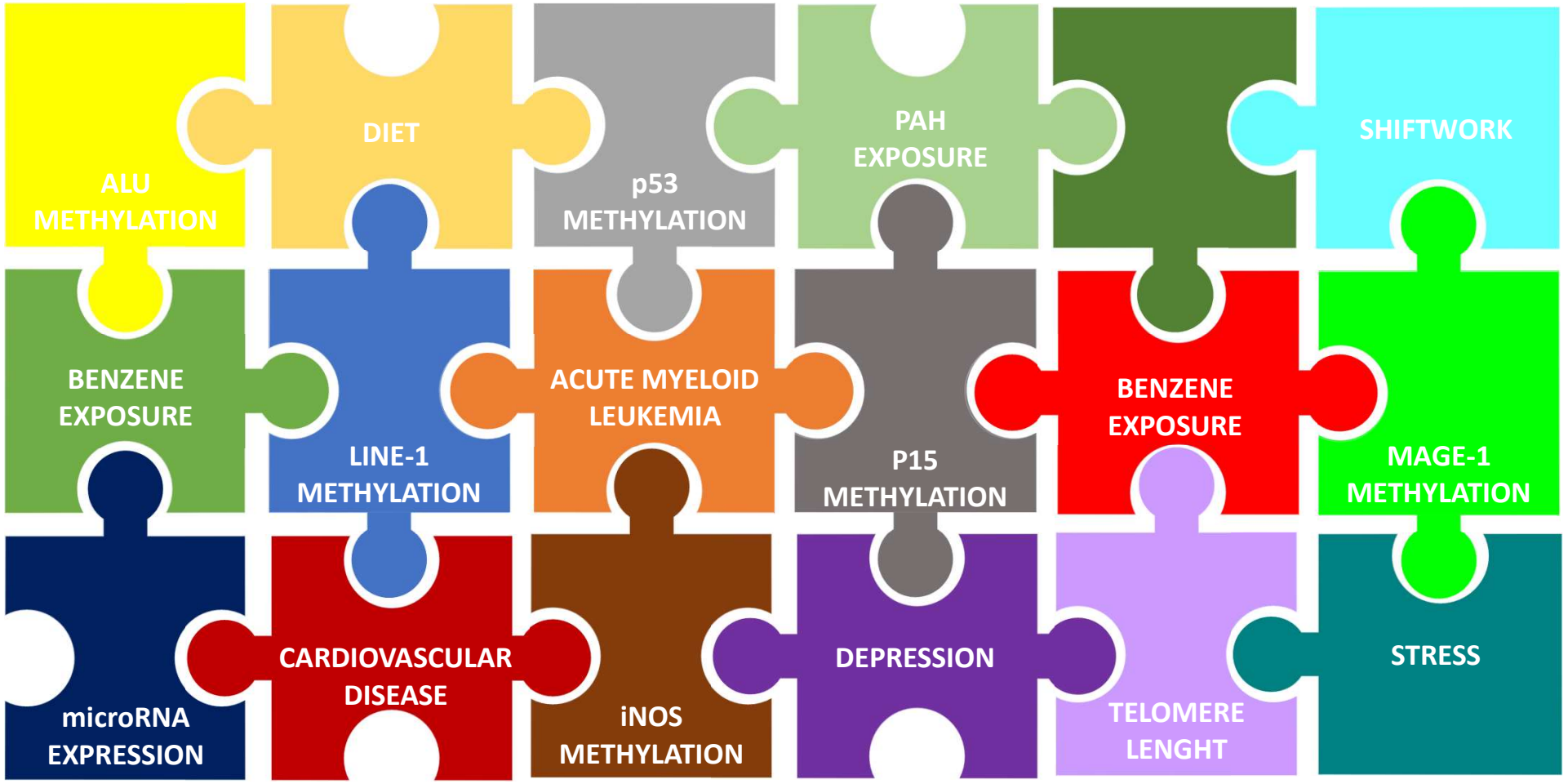
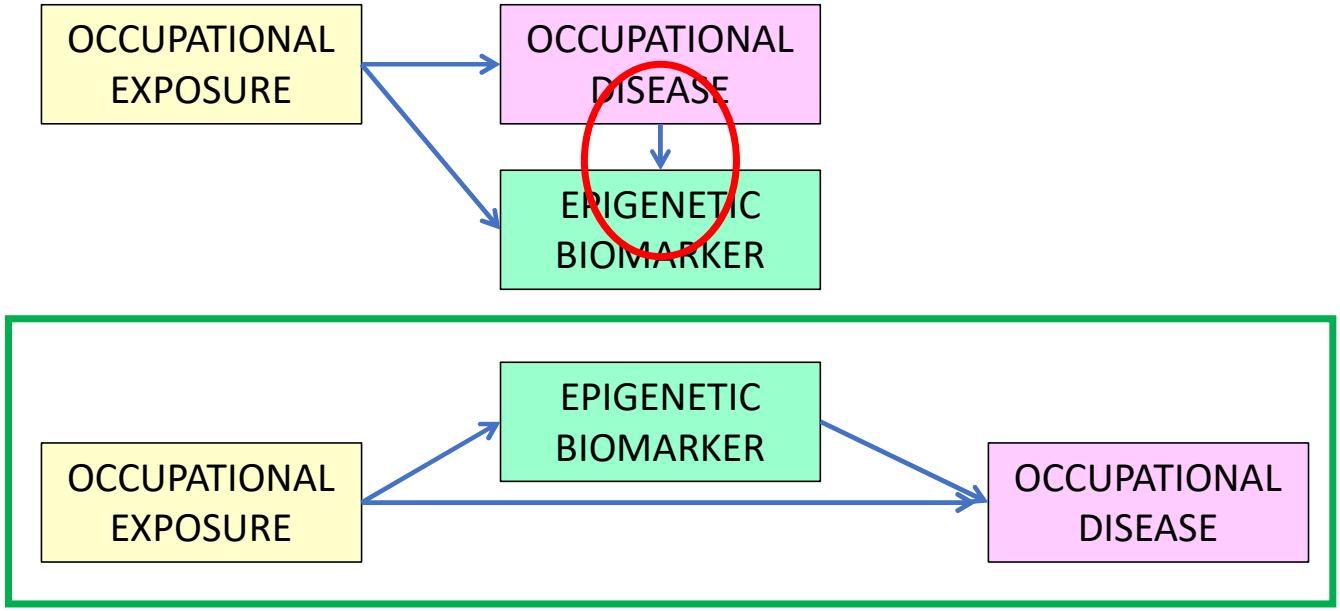

ALU METHYLATION
DIET
p53 METHYLATION
PAH EXPOSURE
SHIFTWORK
BENZENE EXPOSURE
LINE-1 METHYLATION
ACUTE MYELOID LEUKEMIA
P15 METHYLATION
BENZENE EXPOSURE
MAGE-1 METHYLATION
microRNA EXPRESSION
CARDIOVASCULAR DISEASE
iNOS METHYLATION
DEPRESSION
TELOMERE LENGHT
STRESS

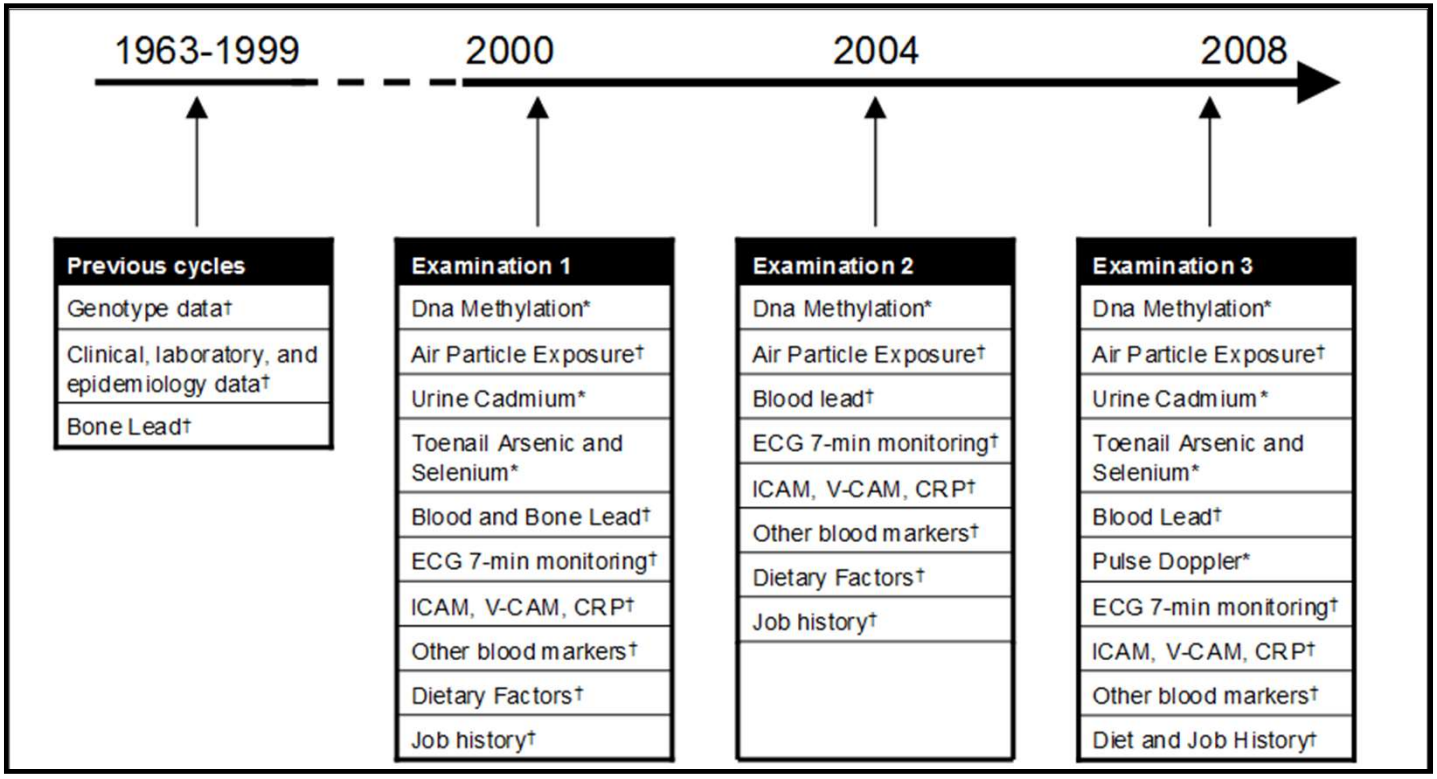


NEED of PROSPECTIVE STUDIES

Normative Aging Study (NAS)

- The Normative Aging Study (NAS) is a longitudinal study established by the United States Department of Veterans Affairs in 1963.
- Most participants are veterans from World War II and the Korean War.
- Participants were male residents and free of chronic disease at the time of recruitment.
- Participants were invited to undergo an in-person examination every 3-5 years.

Normative Aging Study (NAS)



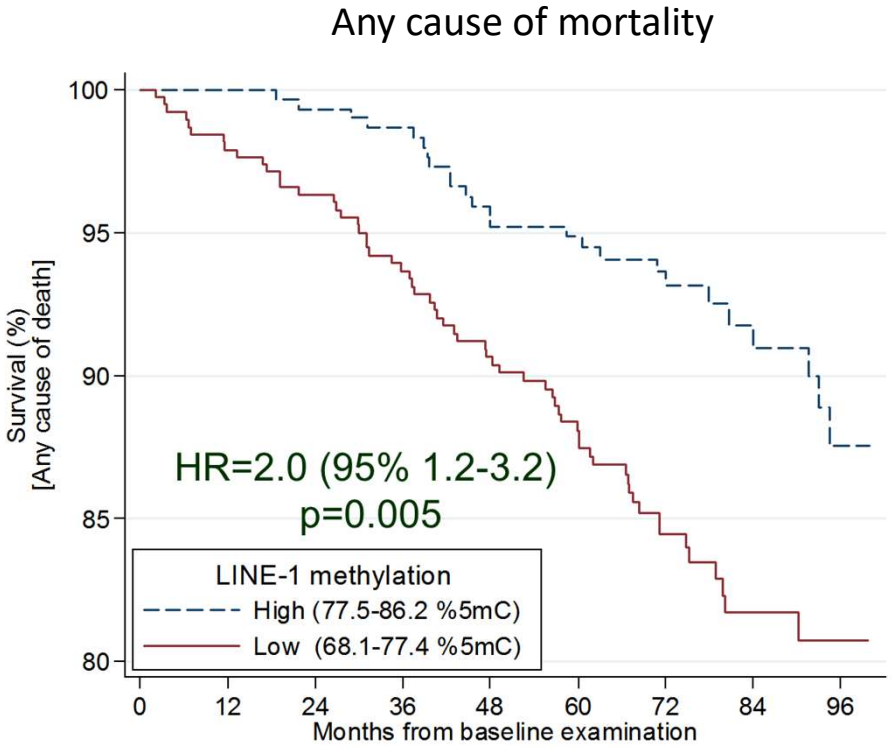
Baseline sample (year 2000) N=742

LINE-1 METHYLATION

Results

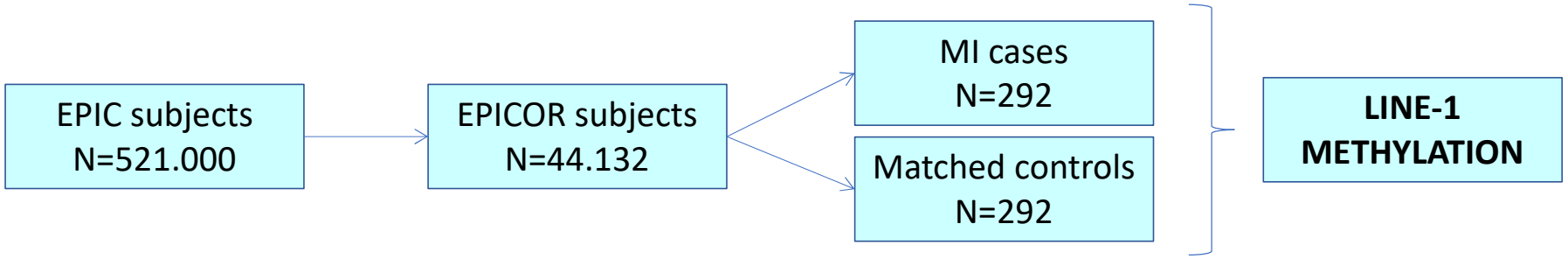
- Individuals with <median LINE-1 methylation show an HR = 3.0 (95% CI 1.3-6.9) of developing cancer (follow-up: mean of 2,894 person-years, 30 cases of cancer).
- Individuals with <median LINE-1 methylation have increased mortality (HR = 3.2, 95% CI 1.3-7.9).

Zhu ZZ, et al. Cancer Causes Control. 2011;22(3):437-47.
Baccarelli A, et al. Epidemiology. 2010 Nov;21(6):819-28.



Italian European Prospective Investigation into Cancer and Nutrition (EPIC) cohort

- The EPIC study is one of the largest cohort studies in the world, with more than half a million (521 000) participants recruited between 1993-1998 across 10 European countries.
- EPIC was designed to investigate the relationships between diet, nutritional status, lifestyle and environmental factors, and the incidence of cancer and other chronic diseases.



Guarrera, S. *et al. Clin Epigenet* **7,** 133 (2015)

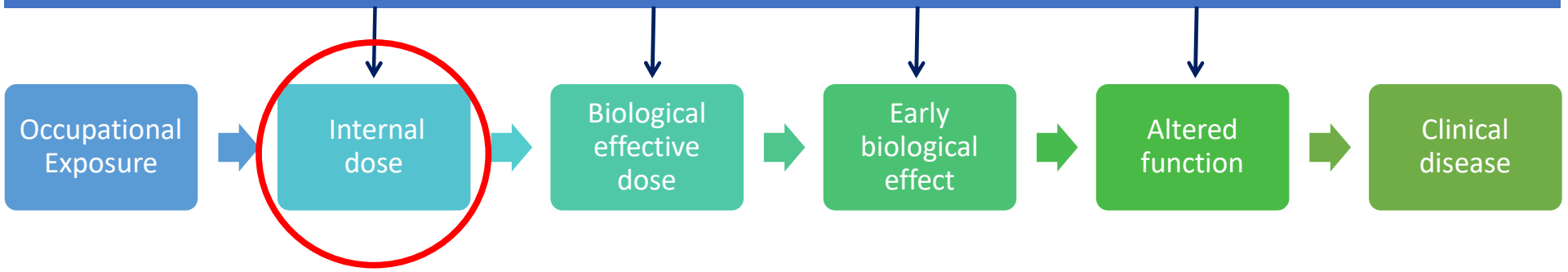
LINE-1 hypomethylation is associated with myocardial infarction (MI) risk

- MI cases had statistically significant LINE-1 hypomethylation as compared to controls (β = −0.511, p-value = 0.0005).
- LINE-1 was hypomethylated in cases with shorter time to disease (meta-analysis, men: $P_{TREND} = 0.0016$, women $P_{TREND} = 0.026$).
- Findings were replicated in independent samples (EPIC-NL):
  - MI risk associated with LINE-1 lower methylation class in EPIC-NL men (OR = 1.95, $P = 0.043$, ref. group above the median).
  - No difference was found in EPIC-NL women (OR = 1.05, $P = 0.850$).
- **DNA methylation profiles improved case-control discrimination and reclassification when compared with traditional MI risk factors only.**

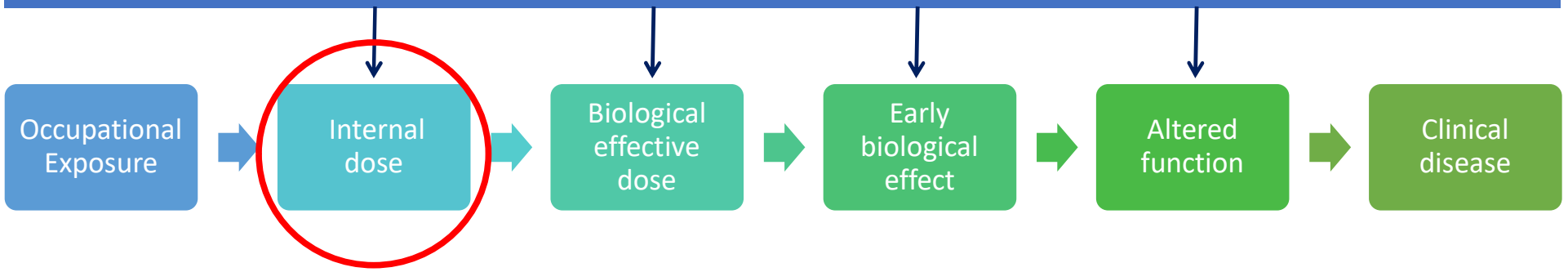
Guarrera, S. *et al. Clin Epigenet* **7,** 133 (2015)

Effect biomarker
or
NOT?

Exposure and Effect Biomarkers

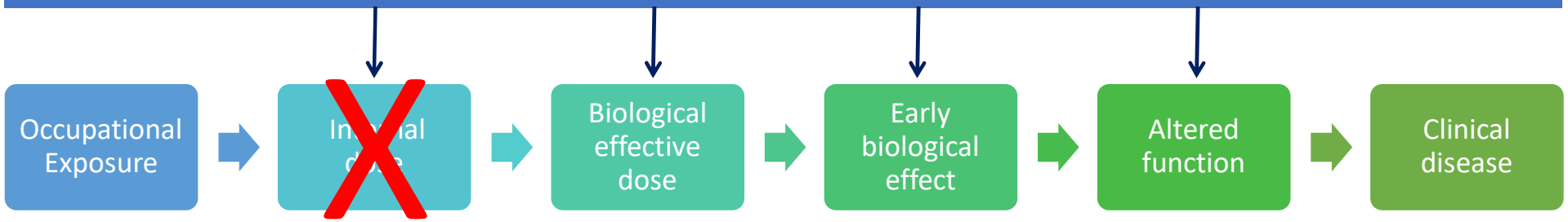


Exposure and Effect Biomarkers



- Often a dose-response is present
- Not specific at all

Exposure and Effect Biomarkers



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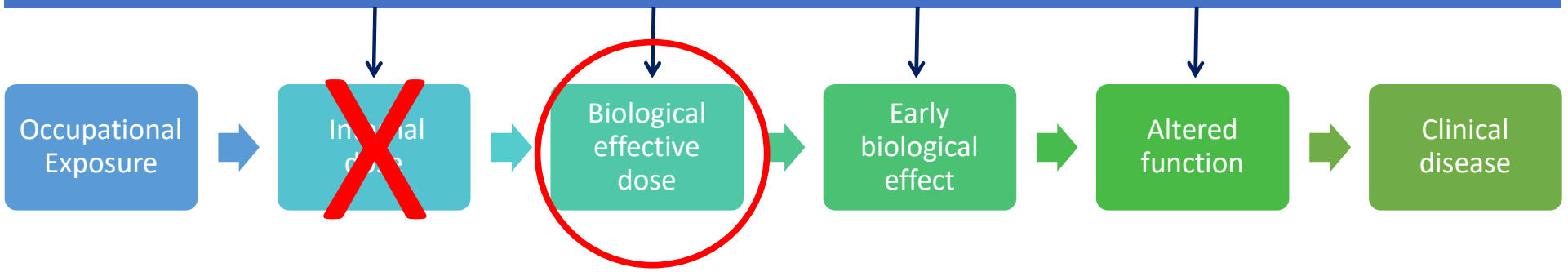

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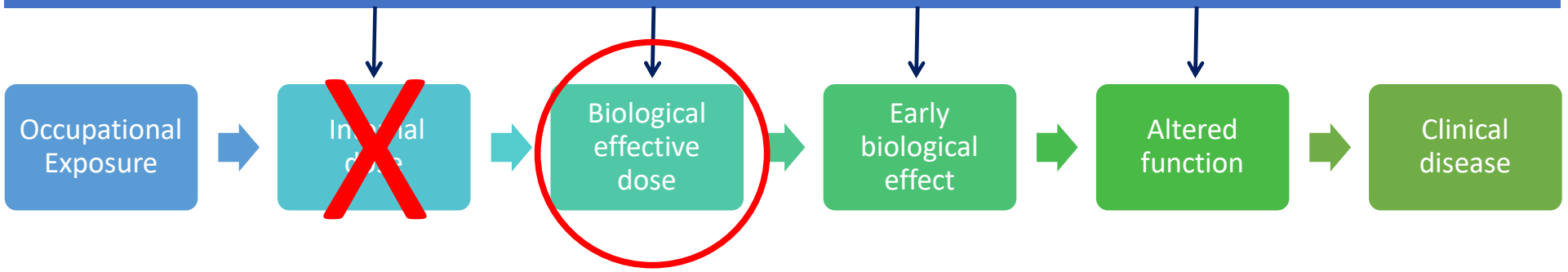
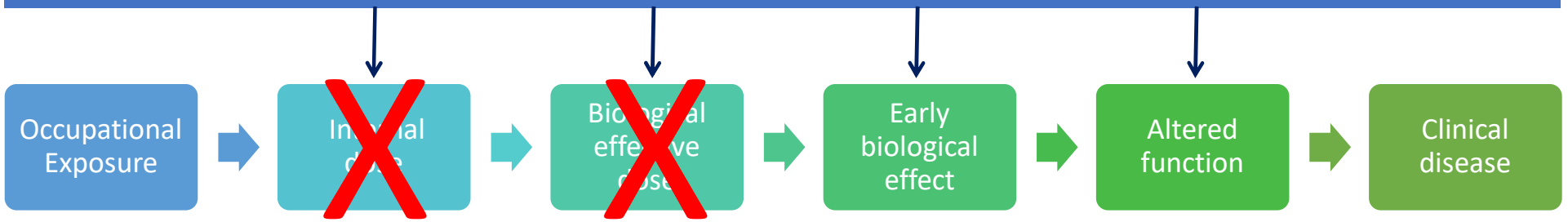


- A threshold of effect is usually not defined

Exposure and Effect Biomarkers



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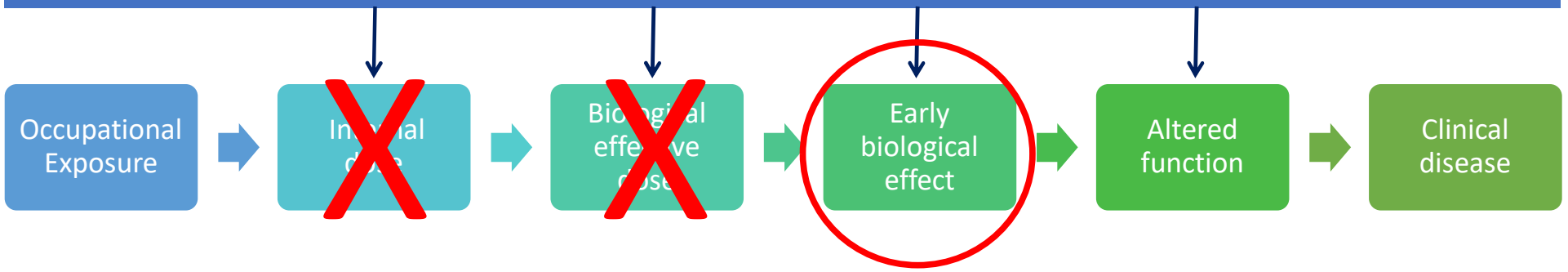

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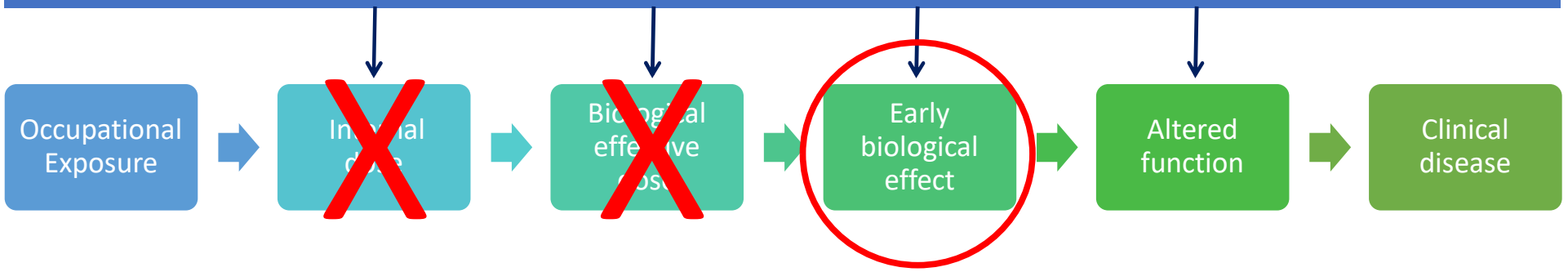
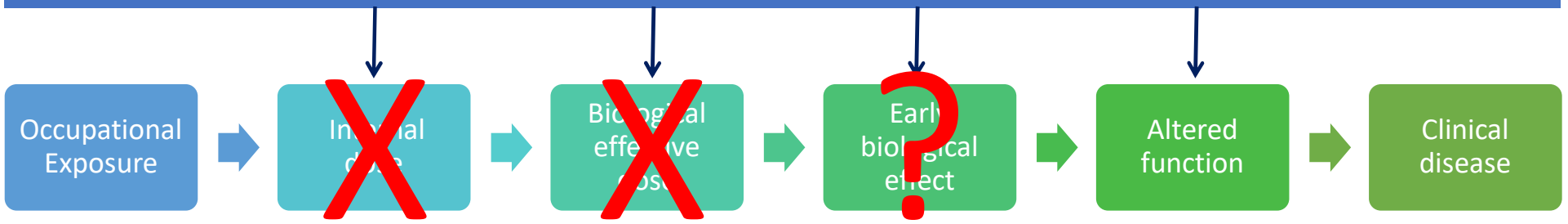


- Biological changes are in common with several exposures
- Not a specific change but rather the response of the organism to changes in its environment, taken as a whole
- It is possible that a single exposure is linked to a PATTERN of modifications

Exposure and Effect Biomarkers



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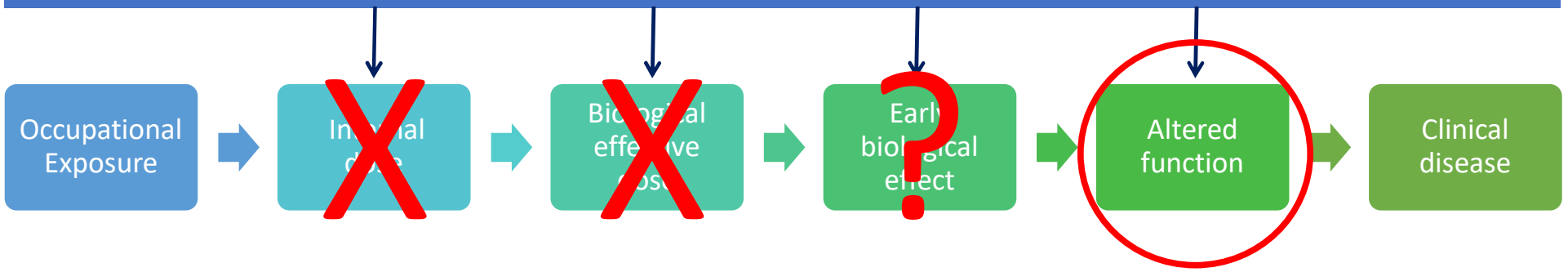

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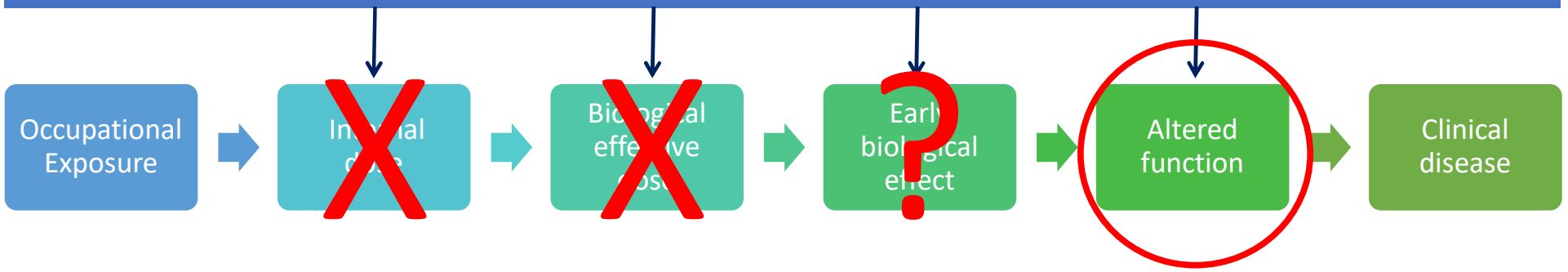
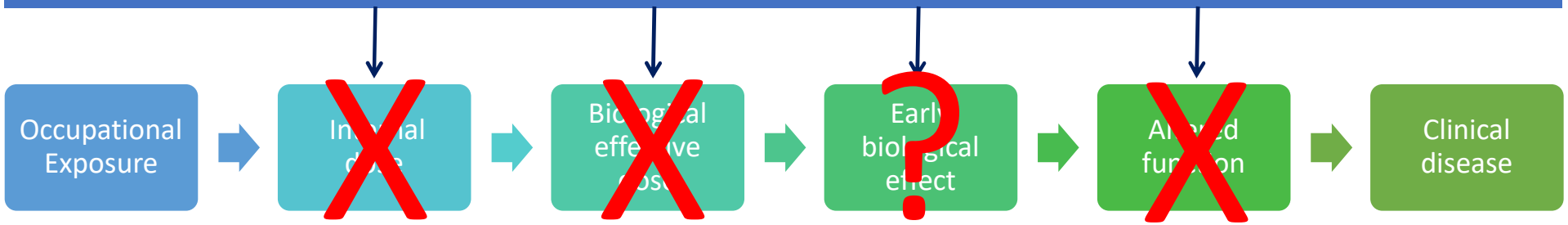


- More than one exposure can converge on pathogenic pathways
- Can be used to estimate a risk of disease, but not to diagnose a disease

Exposure and Effect Biomarkers



Are these markers USEFUL?

TARGETED WORKER HEALTH PROMOTION


Thank
You!