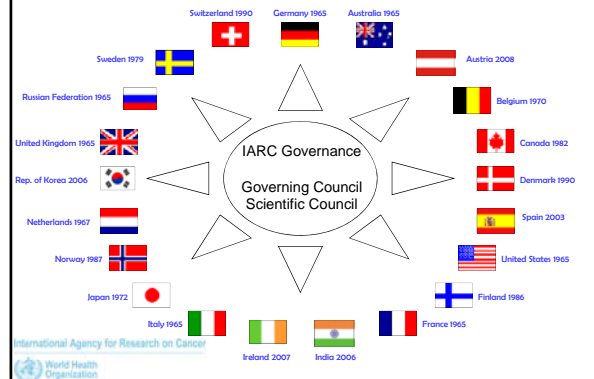


## Complementing the genome with an exposome: the importance of biomarkers of exposure

Dr Christopher P Wild PhD

International Agency for Research on Cancer  
Lyon, France

## IARC's 21 Participating States

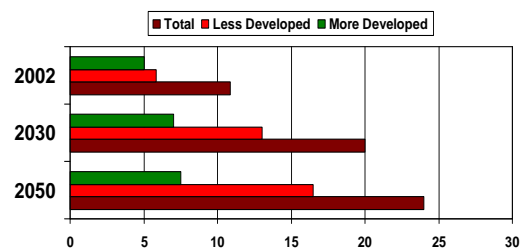


## Complementing the genome with an exposome: the importance of biomarkers of exposure

1. Environment, lifestyle and global burden of cancer
2. Importance of exposure assessment
3. What biomarkers may offer – examples, including mycotoxins
4. Summary - priorities

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## The Growing Global Cancer Burden



In 2008 : 12.4 million new cases; 7.6 million deaths

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IARC, GLOBOCAN 2002

## Socio-demographic influences on cancer burden

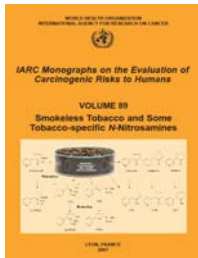
- Population growth - world population estimated to grow from 6.7 billion in 2008 to 8.3 billion by 2030; 4% growth in more developed regions, ~40% in more developing regions
- Ageing - 10 per cent >65 years in 2000, projected to reach 21 per cent in 2050
- Changing lifestyle and exposures – 1% increase per year in incidence

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## Major cancer risk factors

- Tobacco (multiple tumour sites; 30% of cancers in high-resource countries; 1.3 billion people worldwide are smokers)
- Infections (15-20% of cancers worldwide; >25% in developing countries)
- Diet – (relatively little understood concerning how specific nutrients or dietary patterns affect risk)
- Obesity, overweight, physical inactivity - (estimated 1.5 billion people obese by 2015; up to 1/3 of cancers of colon, breast, endometrium, oesophagus and kidney)
- Radiation (ionizing, sunlight)
- Reproductive factors and hormones
- Alcohol
- Occupation
- Environmental pollution

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Recently completed a review of all Group 1 carcinogens in Volume 100 (six parts)

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## The European Partnership for Action Against Cancer

- One third of cancers are preventable – the most cost-effective response
- But 80-90% of cancers have an environmental cause, so the potential for prevention is much higher; *there remains much unknown*
- Increased research on causes of cancer e.g.
  - Diet and metabolism
  - Obesity, physical activity

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## Importance of environmental exposure assessment

- Most major common diseases have an environmental aetiology
- Currently exposure measurement is problematic in many areas, leading to misclassification
- Large prospective cohort studies (e.g. UK Biobank) are predicated on the availability of accurate exposure assessment
- Exposure biomarkers can contribute to several areas in addition to elucidating disease aetiology

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## Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology



- Wild CP (2005) Cancer Epidemiology, Biomarkers and Prevention, 14: 1847-1850.
- Wild CP (2009) Mutagenesis 24: 117-125.

*Uca Pugnax*, the male Fiddler Crab

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## Challenges in characterising the “exposome”

- Scale and complexity: characterisation of life-course environmental exposures, including lifestyle, nutrition, occupation etc.,
- Dynamic: Unlike the genome, the “exposome” changes over time – possibility of critical windows of exposure e.g. in early life
- However, even partial characterisation can bring major benefits

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## Advances in exposure assessment

- Biomarkers
- Geographic information systems
- Personal and environmental monitoring
- Sophisticated questionnaires

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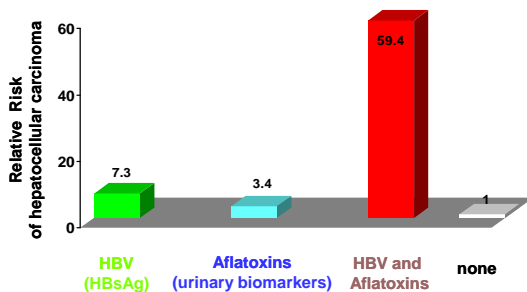
## Exposure biomarkers in population studies – what do they promise?

- Defining etiology
  - Improved exposure assessment – reduced misclassification
  - Identifying susceptible individuals or sub-groups
  - Contributing to biological plausibility

## Exposure biomarkers in population studies – what do they promise?

- Evaluating Interventions
  - Primary and secondary prevention
  - Bio-monitoring e.g. occupational setting
- Hazard and Risk Assessment
  - Mechanistic data (e.g. IARC Monographs)
  - Extrapolation from animal to human
  - Pharmacokinetic-based models

## Interaction between HBV infection and aflatoxins in hepatocellular carcinoma



## Fumonisin and human health

- Produced by *Fusarium spp.*, common contaminants of maize and maize products
- Leukoencephalomalacia in horses and pulmonary oedema in swine; carcinogenic in rodents (liver and renal tumours)
- Linked to oesophageal cancer and neural tube defects in some population studies; FB1: "2B - possibly carcinogenic to humans" (IARC)
- Disrupt complex sphingolipid biosynthesis

## Tortilla Consumption in Mexican women

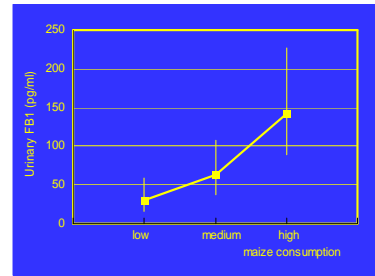


Characteristics	Maize consumption group		
	Low n=24	Medium n=25	High n=26
<b>Tortillas Consumption</b>			
<b>Number of Tortillas consumed each meal Median (range)</b>	2 (1-5)	3 (3-3)	10 (6-16)
<b>No. of meals/per day when tortillas consumed Median (range)</b>	<1 (0-0.4)	2.5 (2.5-2.5)	2.5 (2.5-6)

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## Urinary FB1 in relation to maize consumption in Mexican women

(Gong et al., Cancer Epidemiol. Bio. Prev., 2008)



Samples above detection limit (%)

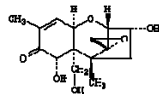
45

80

96

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## Deoxynivalenol – the aflatoxin of the north?



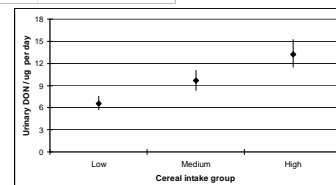
- Produced by *Fusaria spp.*; common contaminant of cereals
- Vomiting, feed refusal, weight loss, immuno-modulation in animals and induces IgA nephropathy in mice
- Alters cell signalling and cytokine expression (e.g. MAPK)
- Linked to GI poisoning in China and India
- Urinary biomarker applied to the UK National Diet and Nutrition Survey samples

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## DON exposure in relation to cereal consumption

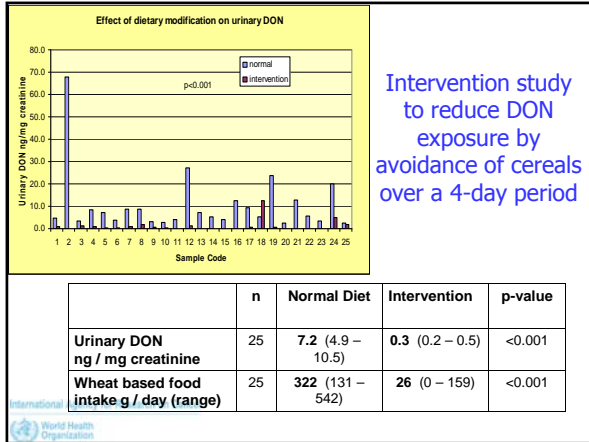
Group	Cereal Intake g/day (range)	DON µg/day Mean (range)
Low	107 (88-125)	6.6 (5.8-7.6)
Medium	179 (162-195)	9.6 (8.4-11.0)
High	300 (276-325)	13.1 (11.5-15.1)

**DON was detected in 296/300 (98.7%) of the urines**



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Data are adjusted for sex, age and BMI; p for trend <0.001, adjusted R<sup>2</sup> =0.182.



### Biomarkers and classification of carcinogenicity

Carcinogen	Discovered	IARC classified Group 1
<i>Helicobacter pylori</i>	1983	1994
Aflatoxins	1963	1987 (Suppl. 7) and 1993
Fumonisin	1988	Currently Group 2B

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- ### Validation and application
- A plea for validation – difficult to find support for, but essential for progress
  - An integral part of method development should be the consideration of throughput, cost and applicability to biobank samples
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- ### Complementary emphasis in exposure biomarkers
- First generation exposure biomarkers tended to focus on a classical mutagen – carcinogen model of carcinogenesis (*metabolites, adducts, chromosomal alterations, somatic mutations*)
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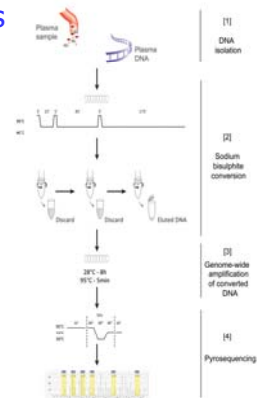
## Biomarkers in relation to other mechanisms of carcinogenesis

- Epigenetic changes (promoter methylation, histone acetylation, microRNA)
- Altered gene, protein or metabolite levels

Potential application to “exposures” such as nutrition, obesity, physical activity

## Epigenetic biomarkers – applicability to population studies

Quantitative analysis of DNA methylation after whole bisulfite amplification of a minute amount of DNA from body fluids  
(Vaissiere *et al.*, *Epigenetics*, 2009)



## Epigenetic biomarkers – applicability to population studies

- Detection of stable miRNAs in plasma and serum – differences by disease status (Mitchell *et al.*, *PNAS* 105: 10513, 2008; Chen *et al.*, *Cell Res.*, 18: 997, 2008)
  - Cell and tissue specific expression
  - Stable in biological fluids such as plasma and serum
  - PCR based assays available
  - Profiling a small number may provide discrimination
  - Genetic variations in miRNA processing genes and in miRNA binding sites may confer genetic susceptibility
  - **Functional information is vital**

## Can “omics” help improve exposure assessment?

- Do specific exposures, or categories of exposure, alter the expression of specific groups of genes, proteins or metabolites (“exposure fingerprint”)?
- How do such alterations relate to dose?
- How stable are the alterations over time?
- How do potential confounding factors affect the association between exposure and “omics” biomarkers

## Transcriptomics and exposure assessment

(see Wild CP, *Mutagenesis* 24: 117-125, 2009)

- **Smoking** – Lampe et al., *CEBP*, 13: 445-453, 2004; van Leeuwen et al., *Carcinogenesis*, 28: 691-697, 2007
- **Benzene** - Forrest et al., *EHP* 113: 801, 2005
- **Arsenic** – Fry et al., *PLoS Genet.*, 3: 2180-2189, 2007; Wu et al., 111: 1429-1438, 2003
- **Metal fumes** – Wang et al., *Env. Health Persp.*, 113: 233-241, 2005
- **Air pollution** – van Leeuwen et al., *Mutat. Res.*, 600: 12-22, 2006

## Metabonomics and population studies

- Connects molecular events to those at the macro level
- Applicable to blood and urine samples
- LC-mass spectrometry methodology affordable and of requisite throughput
- Demonstrated applicability to studies of diet (Solanky et al., *Anal. Biochem.*, 323: 197-204, 2003; Holmes et al., *Nature*, 453: 396-400, 2008)

## Problems in comparisons of “omics” data in poorly designed studies

See Potter JD *Trends in Genetics*, 19: 690-695, 2003

- Unmeasured confounding by lack of information on age, sex and other exposures
- Bias through differences in sample processing
- Selection bias through sampling procedures
- High costs leading to one-off or small-scale studies

## Temporal application of exposure biomarkers in cancer epidemiology

← Exposure → Disease

Peri-natal Childhood Adolescence Adult

Birth cohort

Adult cohort

Case-control study

↑ Timing of exposure measurement

Carcinogen metabolites  
DNA/protein adducts  
Cytogenetic alterations

Mutation spectra  
Antibodies

## Early life exposure and cancer risk

- Observational studies linking early life exposures to disease later in life
- Foetal programming; adaptive response - indications of alterations in the epigenome
- Vulnerability of children to environmental exposures
- Reported rise in childhood cancer rates (see Steliarova-Foucher et al., Lancet 364: 2097, 2004)

## Early life exposure and cancer risk - opportunities

- Mother:child birth cohorts – need for international cooperation
- Mechanism-based biomarkers to relate exposure to disease – a necessity?

## Sub-Saharan Africa

- 4.5 million deaths in children under age 5 annually
- 175 child deaths (<5 yrs) per 1000 live births (c.f. 6 per 1000 in industrialized nations)



Under-nutrition and growth faltering is an underlying cause of 50% of deaths in children <5 years age (Black et al., Lancet, 2003)

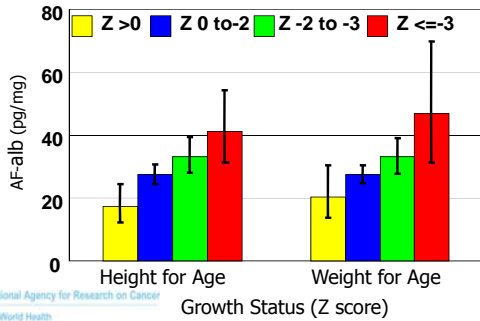


## Aflatoxin, weaning and effects on growth in young children in Benin and Togo

- Sixteen villages in four agro-ecological zones
- 479 children (age 9 months - 5 years)
- Aflatoxin-albumin in blood
- Anthropometry

Gong et al., Brit. Med J. 2002

## Exposure to aflatoxin associated with impaired growth



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## Longitudinal study of aflatoxin exposure and child growth in Benin

Subjects: 200 children, aged 16-37 months from four villages, two high, two low aflatoxin exposure

Time:	February	May/June	October
Survey:	1	2	3
Serum AF-alb:	X	X	X
Anthropometry:	X	X	X
Questionnaire:	X	X	X

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## Longitudinal Study of Aflatoxin Exposure and Child Growth in Benin

Gong et al., *Environ. Health Perspec.* (2004) 112, 1334-1338

Aflatoxin Exposure Group	Mean AF-alb over 8 months Height increase (cm)	
	Unadjusted	Adjusted <sup>a</sup>
lower quartile	4.9 (4.5,5.3) <sup>c</sup>	5.9 (5.2,6.6)
mid-lower quartile	4.4 (4.1,4.7)**	5.3 (4.8,5.9)
mid-upper quartile	4.1 (3.8,4.5)**	4.8 (4.4,5.2)
upper quartile	4.1 (3.8,4.5)**	4.2 (3.9,4.6)

200 children, aged 16-37 months followed over 8 months  
<sup>a</sup>Adjusted for age, height, weaning status, mothers SES and village.  
<sup>c</sup>Data labelled \* are significantly different to \*\*.

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## Activation of inflammation/NF-κB signalling in infants born to arsenic-exposed mothers

Fry et al., *PLoS Genetics*, 3: 2180-2189, 2007

- 32 pregnant women in Thailand in high and low areas of arsenic exposure
- Toenail analysis of arsenic; cord blood for microarray gene expression
- Expression signatures highly predictive of prenatal arsenic exposure; genes related to stress, inflammation, metal exposure and apoptosis

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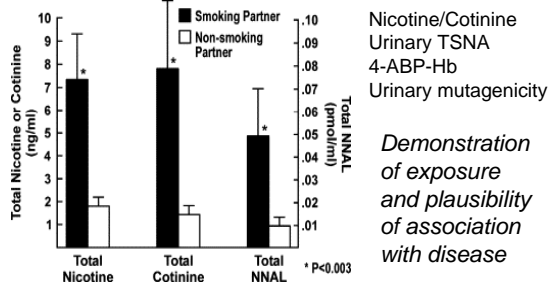
Are there critical windows of exposure during which exposure to environmental risk factors is most relevant?

The public health relevance of an exposure should be considered in relation to all its adverse health effects

## Biomarkers and Biological Plausibility

- Demonstration of exposure
- Evidence for a plausible mechanism

## Demonstration of exposure – environmental tobacco smoke

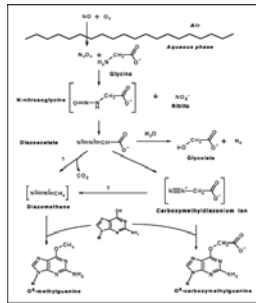


## Red Meat and Colon Cancer Risk: biomarkers and biological plausibility

- Red and processed meat is associated with increased colorectal cancer (CRC); one hypothesis is that this is due to heterocyclic amines (HCA)
- However, white meat contains HCA but is not associated with CRC risk
- Studies of the N-acetyl gene required for HCA activation and CRC are equivocal
- Red but not white meat stimulates endogenous intestinal N-nitrosation in humans

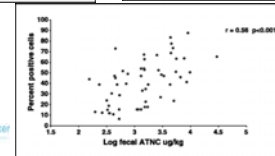
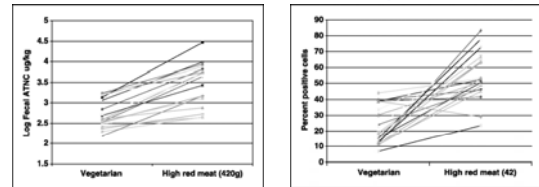
## Red Meat and Colon Cancer Risk: biomarkers and biological plausibility

- Volunteers in metabolic suite
- Fed high (420g) red meat, vegetarian and high red meat, high-fibre diets for 15 days in randomized cross-over trial
- Tested whether total faecal N-nitroso compounds and O6-carboxymethylguanine adducts in colon DNA were associated with red meat diet



Lewin et al., Cancer Res 2006

## Red Meat and Colon Cancer Risk: biomarkers and biological plausibility

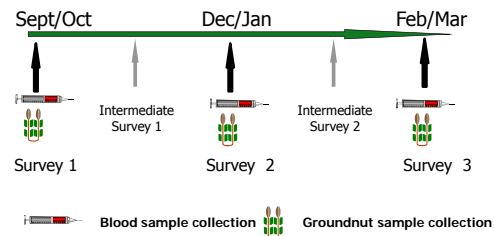


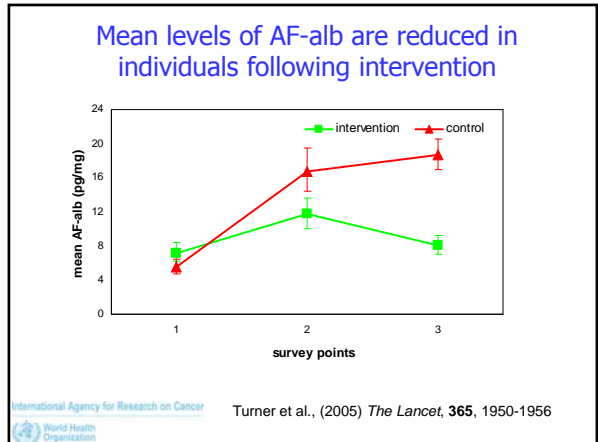
## Biomarkers and intervention studies

- Proof of concept (e.g. anti-oxidants, induction of detoxification enzymes, avoidance of exposure)
- Surrogate (earlier) outcome
- Human experimental studies e.g. dietary modulation

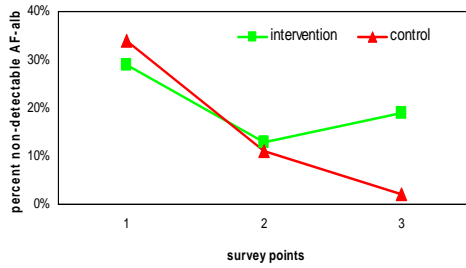
## Biomarkers and intervention studies – aflatoxin in subsistence farms in Guinea

20 Villages (10 intervention, 10 control), 30 subjects per village





### Intervention increases the number of individuals with non-detectable blood AF-alb



International Agency for Research on Cancer World Health Organization Turner et al., (2005) *The Lancet*, **365**, 1950-1956

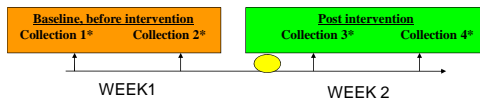
### Reducing fumonisin in maize in the Eastern Cape, South Africa by hand sorting and washing with PROMEC Unit, MRC SA



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### Reducing fumonisin in maize in the Eastern Cape by hand sorting and washing

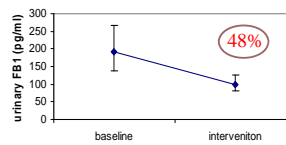
Trained 22 women to perform hand sorting and washing on the maize prior to cooking; measured the reduction of fumonisin exposure after intervention using the urinary FB1 biomarker.



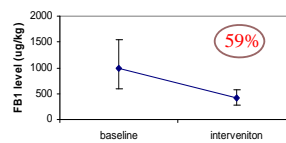
● : Intervention training on Wednesday morning  
 \*Collection 1-4: maize, plate-ready maize meals, morning urine and food intake by 24-hr recall questionnaires

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Urinary FB1 geometric mean levels before and after intervention



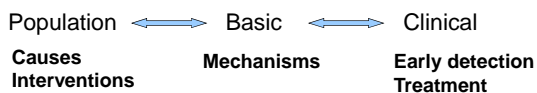
FB1 geometric mean levels in cooked maize meal



### Impact of intervention on food and urinary levels of FB1 in Eastern Cape, South Africa

Gong, van der Westhuizen et al., unpublished

## “Common soil” of mechanistic research



### A plea for two-way translation

## Future challenges, future perspectives

- Improve the coverage and quality of data on the global cancer burden
- Invest in exposure biomarkers to complement genetic analysis for large/expensive prospective cohort studies to fulfil their promise
- Apply new methodologies (e.g. metabonomics) and knowledge of mechanisms (e.g. epigenetics) to population-based investigations of environment, lifestyle and cancer

## Future challenges, future perspectives

- Prioritize studies of biological plausibility in establishing aetiology, particularly in cases of modest risk elevation such as diet and cancer
- Early life exposures merit consideration in the context of mother:child cohorts and related biobanks
- Prioritize research into how to implement prevention strategies

## Acknowledgements



### Collaborators

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