

Biomarkers of exposure to environmental and endogenous leukaemogens

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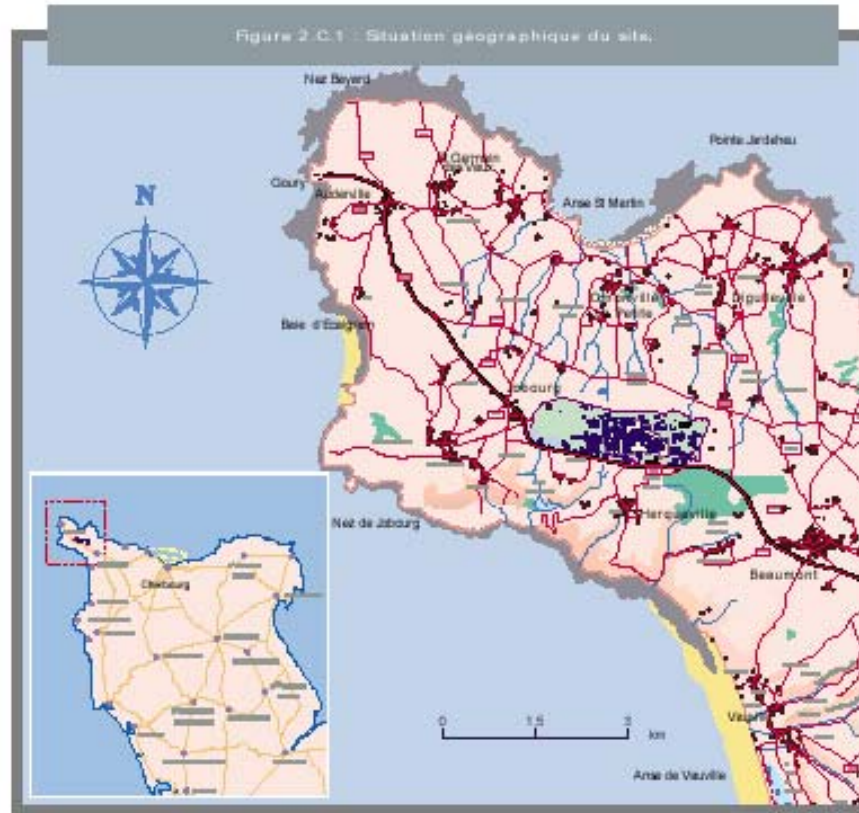


September 2004

Children with Leukaemia

Introduction

- Most cancers are preventable
- DNA damage appears to be involved in carcinogenesis
- How can we put these two facts together to make progress in the battle against cancer?



- Photo 2.C.1 : Paysage maritime



- Photo 2.C.2 : Paysage rural et industriel

“Nobody wants dirt on their DNA”

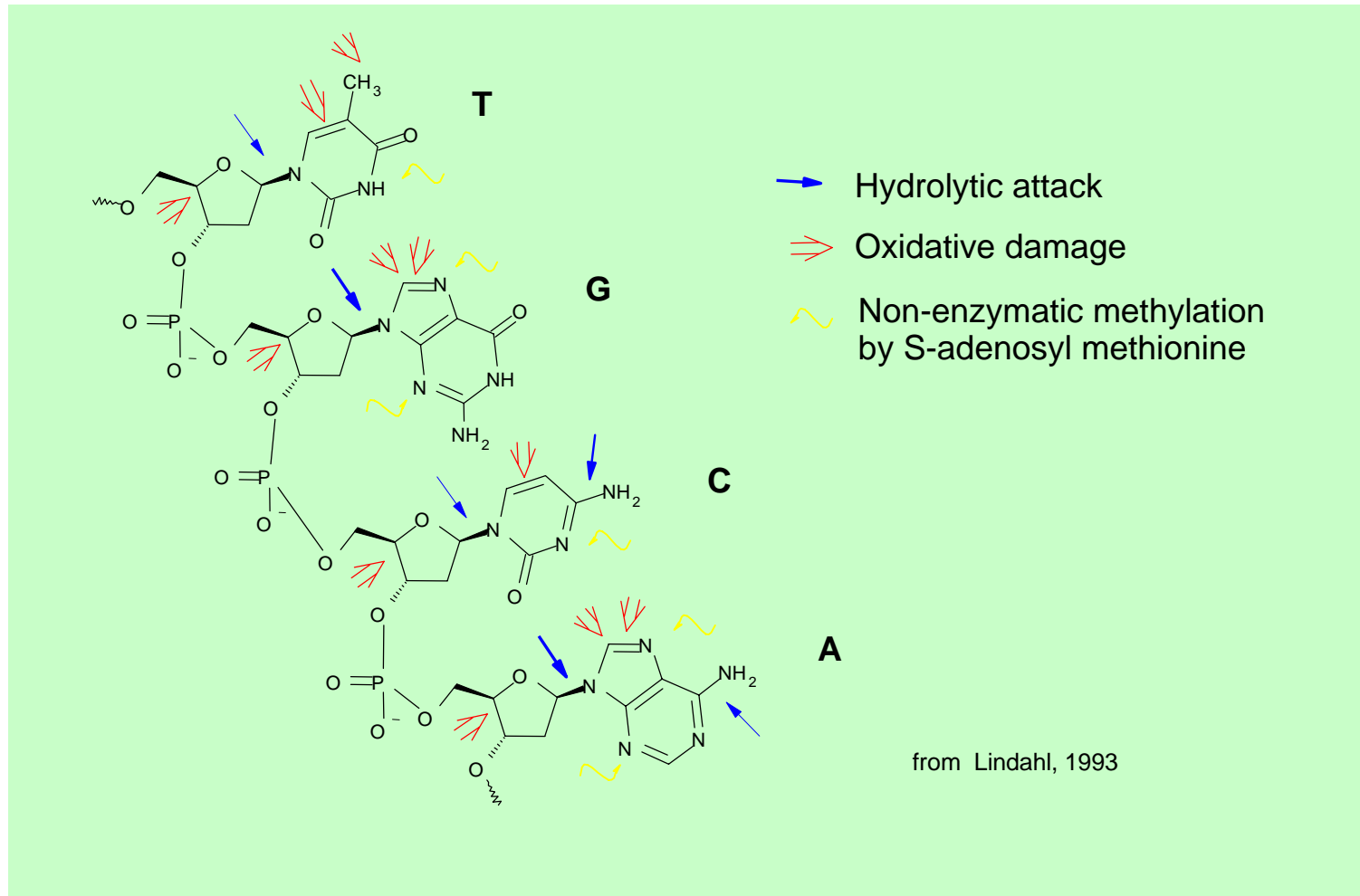
Anon.

Stability of DNA

- “Although DNA is the carrier of genetic information, it has limited stability”
- “All biological macromolecules spontaneously decompose”

Lindahl, *Nature*, 1993, 362, 709

Chemical instability of DNA

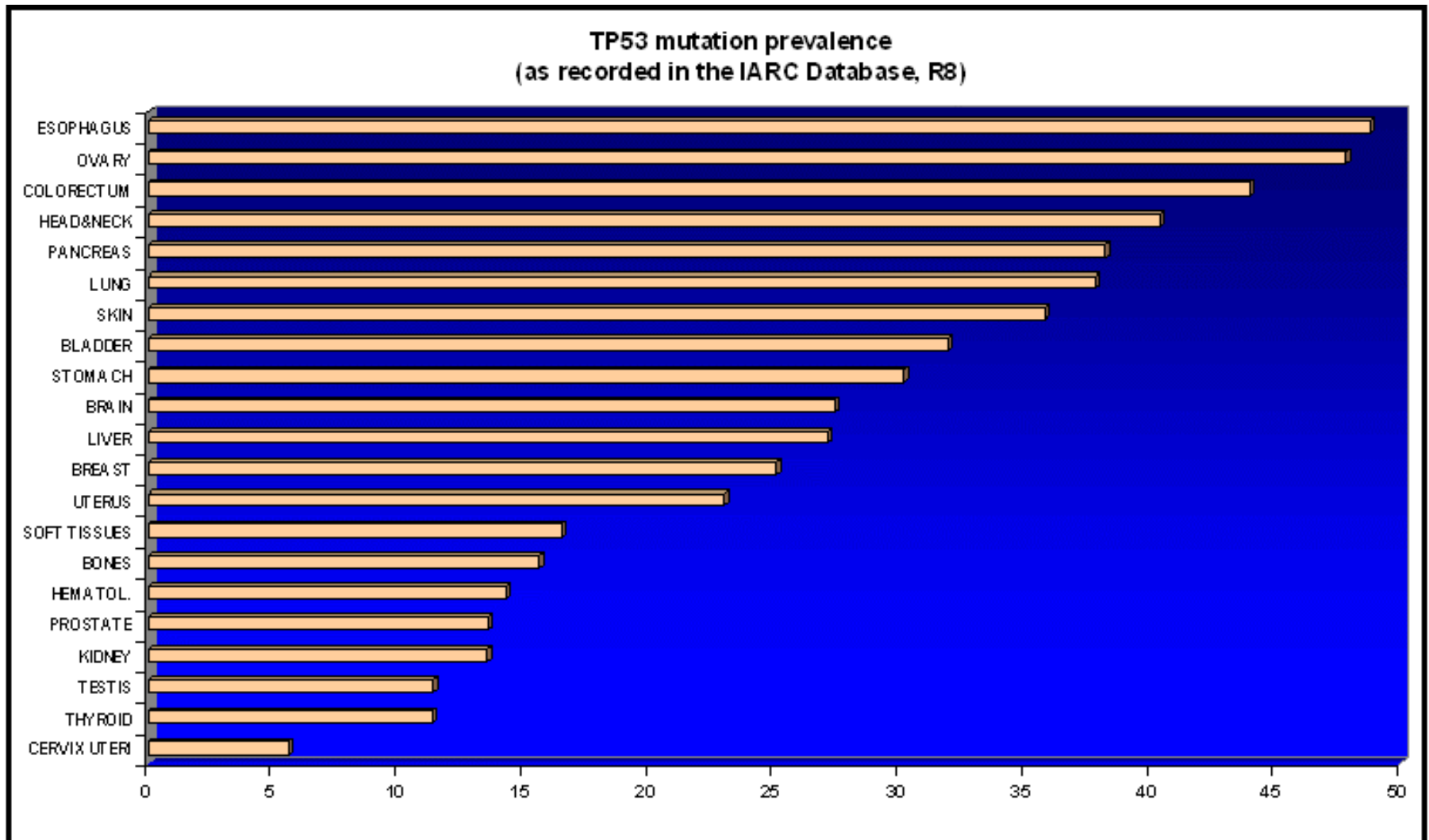


How relevant is DNA damage to cancer?

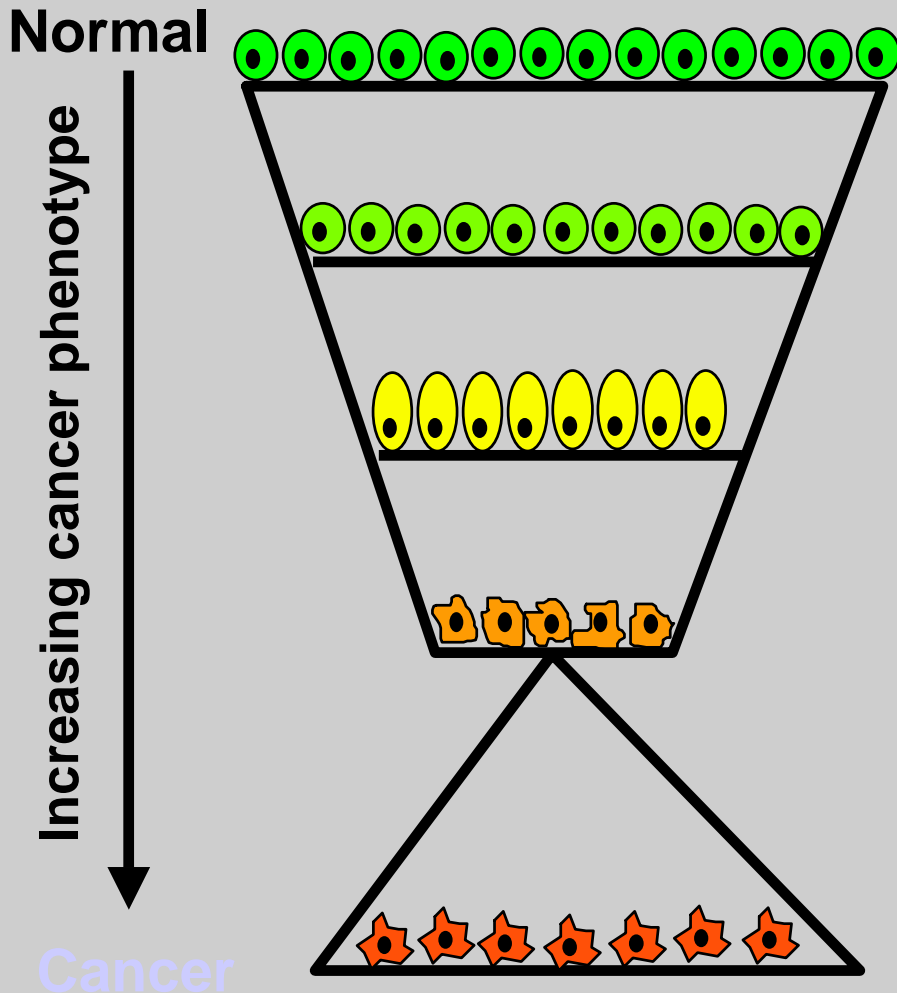


- The human genome is subject to thousands of damage events per day per cell
- There are many reactive intermediates arising from cellular physiology (e.g., ROS, RNS)
- Potent evidence for the importance of DNA damage in carcinogenesis comes from the observation that the p53 gene is mutated in up to 50% of human cancers

Prevalence of p53 mutations



Biomarkers of exposure and risk



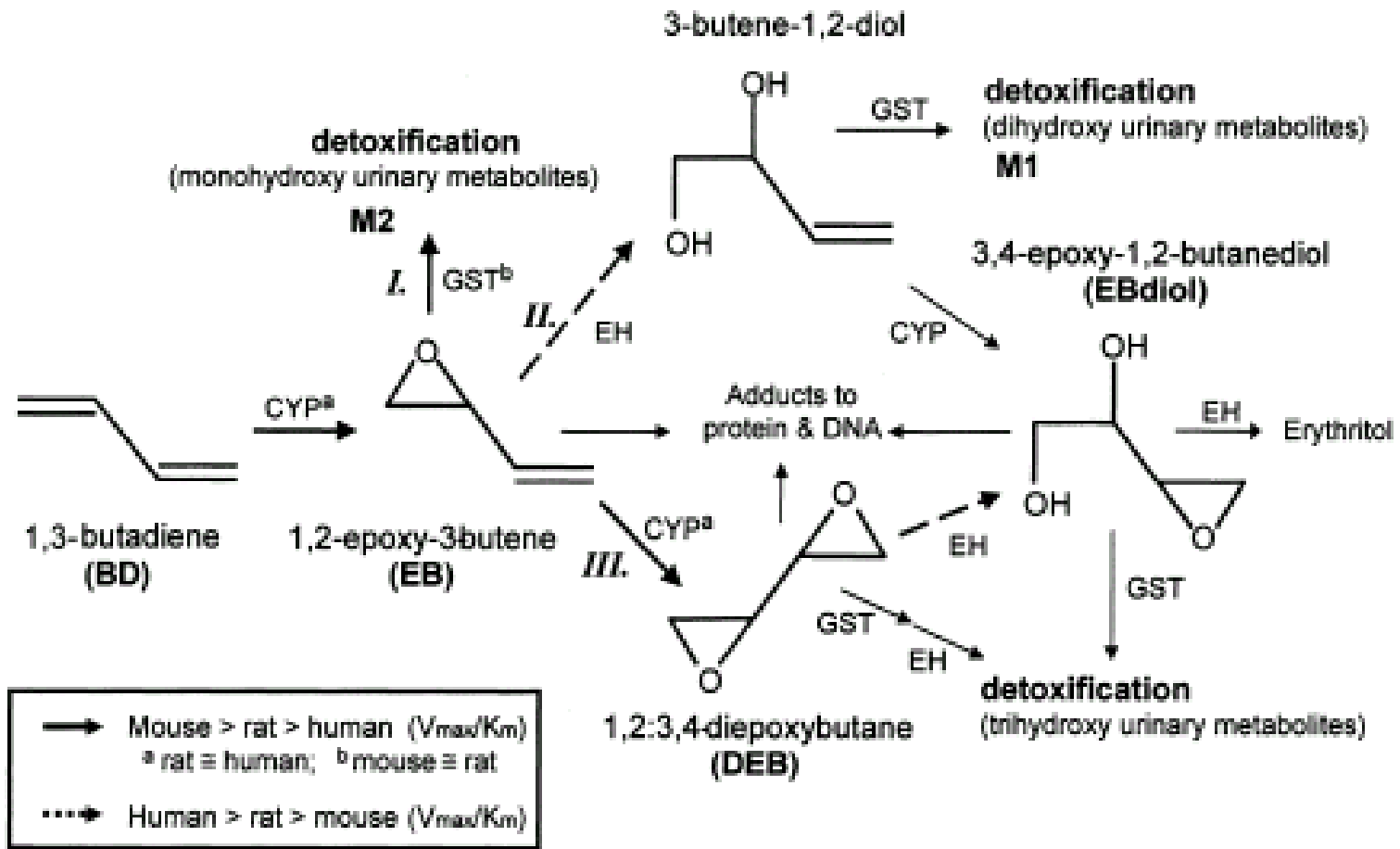
Measures
of
exposure
or early
biological
effect

Indicators
of risk

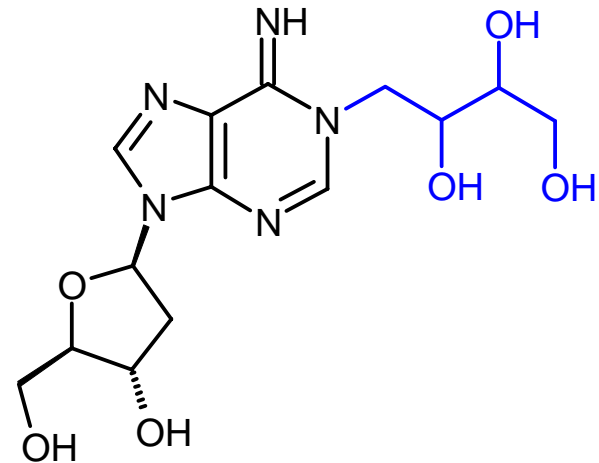
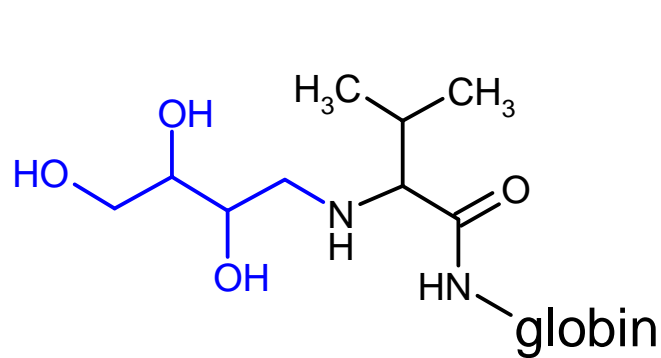
1,3-Butadiene

- Evidence of the carcinogenic hazard of 1,3-butadiene comes from two main sources: firstly, studies of human populations exposed in the workplace and, secondly, from investigations carried out in laboratory animals
- In 1999 an IARC working group concluded that 1,3-butadiene was probably a human carcinogen (group 2A)
- In the absence of compelling human epidemiology that fact that 1,3-butadiene displayed a similar metabolic pattern in experimental animals as humans and that both DNA and protein adducts were detected in exposed humans provided key pieces of evidence to support the evaluation

1,3-Butadiene



DNA and protein adducts



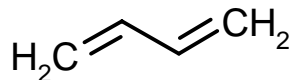
1,3-butadiene forms characteristic adducts with both globin and DNA

1,3-Butadiene

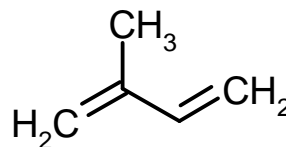
- In the UK an environmental air standard for 1,3-butadiene has been set at 1 ppb using an NOAEL of 1,000 ppb
- DNA adducts to 1,3-butadiene can be detected at $>1,000$ ppb but the relationship between adducts and biological effect remains unclear
- However, detection of 1,3-butadiene adducts at levels of <10 ppb is difficult

Isoprene - a possible endogenous exposure

- Isoprene is a ubiquitous natural precursor to many important biological molecules and is structurally very similar to 1,3-butadiene and undergoes similar metabolism
- Humans exhale isoprene



1,3-butadiene

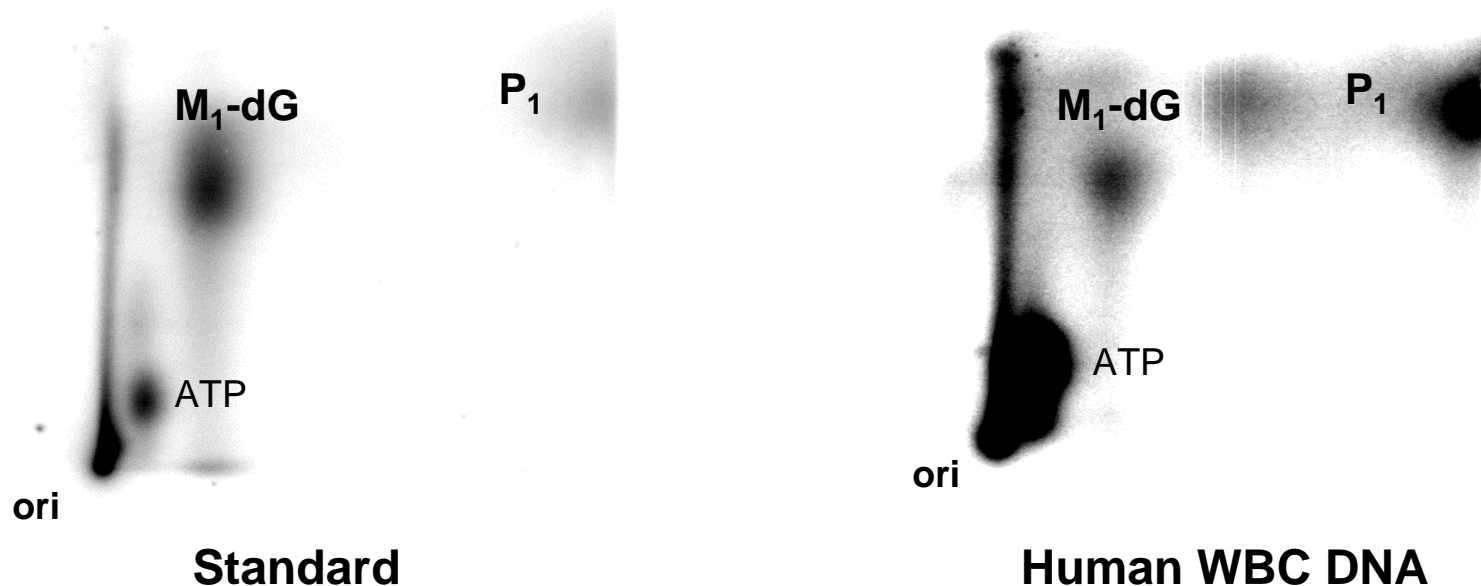


Isoprene

Increasing sensitivity

- The ^{32}P -postlabelling assay developed by Randerath and Randerath and colleagues revolutionised the detection of DNA damage
- The identity of the adduct(s) did not need to be known *a priori*
- One modified base in a billion (10^9) could be detected
- A number of environmental genotoxicants have been detected (PAH, tobacco smoke)

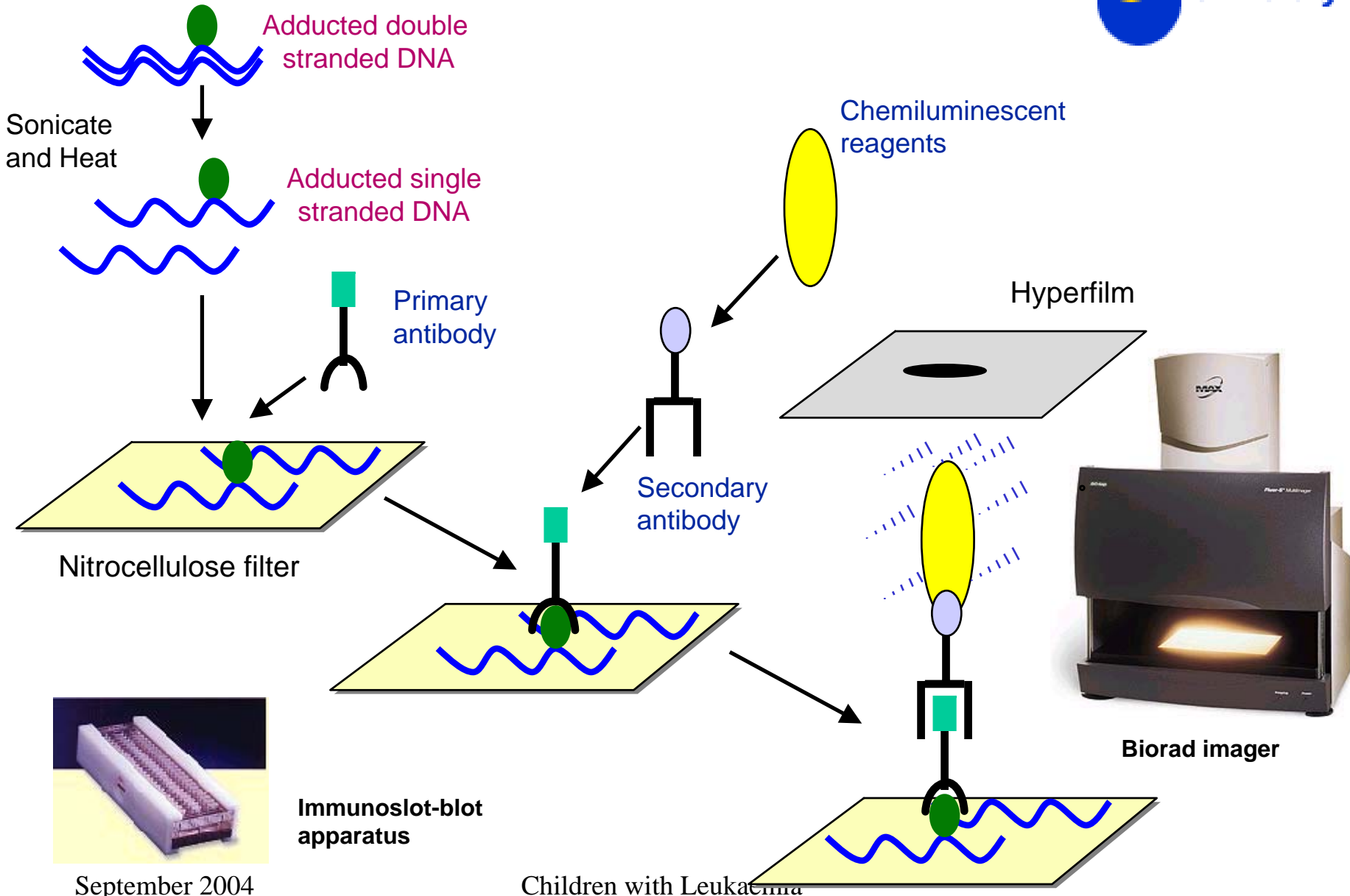
^{32}P -postlabelling



Adducts are separated as 3',5'-diphosphates with ^{32}P introduced into the 5' position

Leuratti et al., 1998
Children with Leukaemia

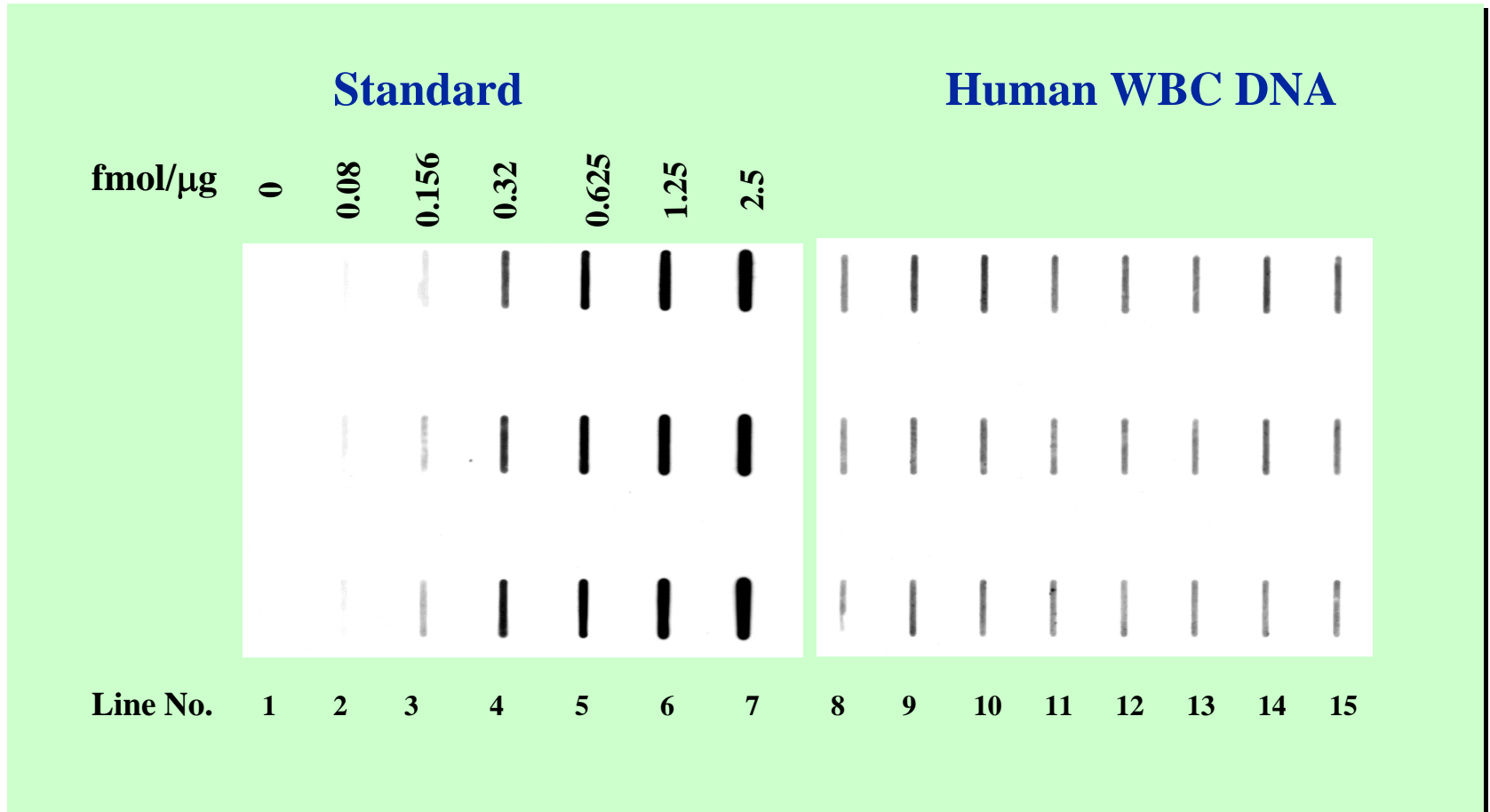
Schematic representation of the immunoslot-blot procedure



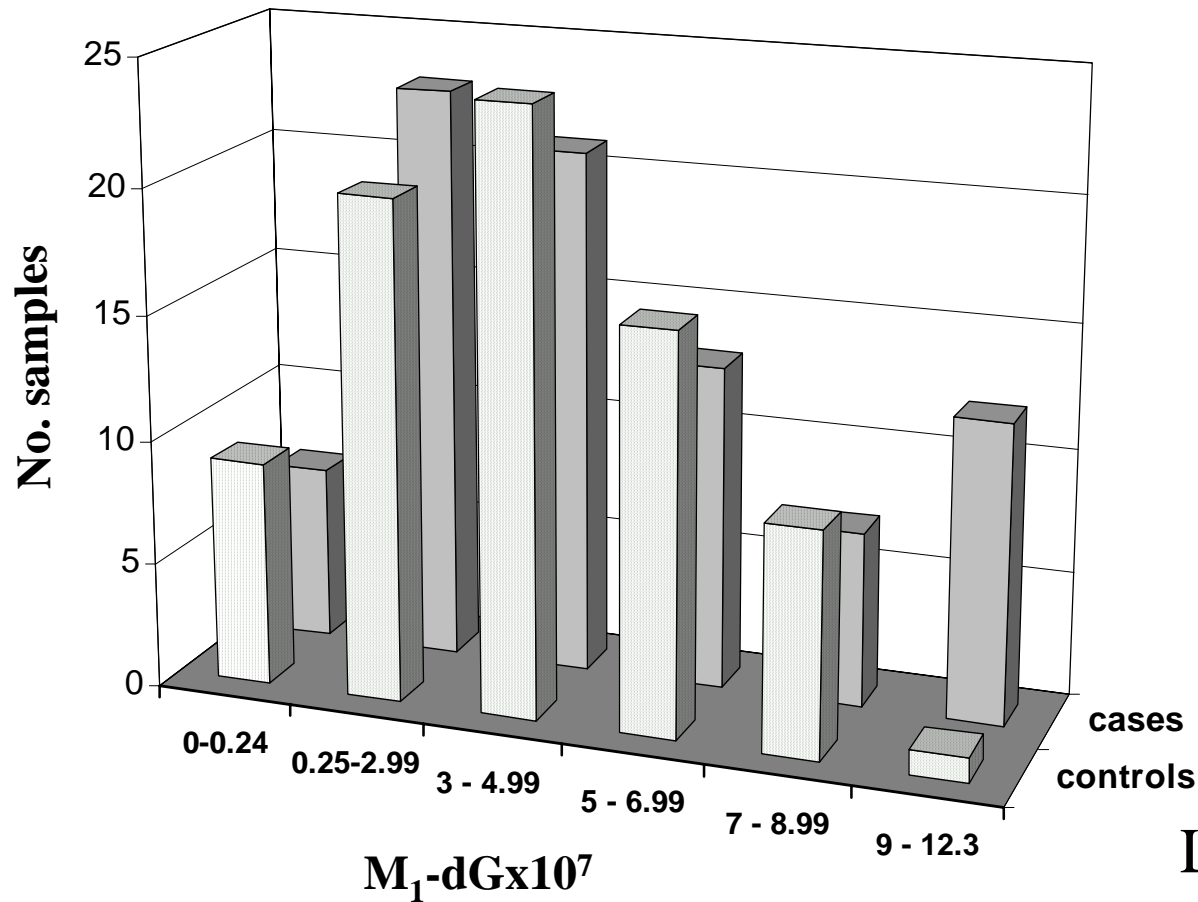
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Children with Leukaemia

M₁dG immunoslot blot

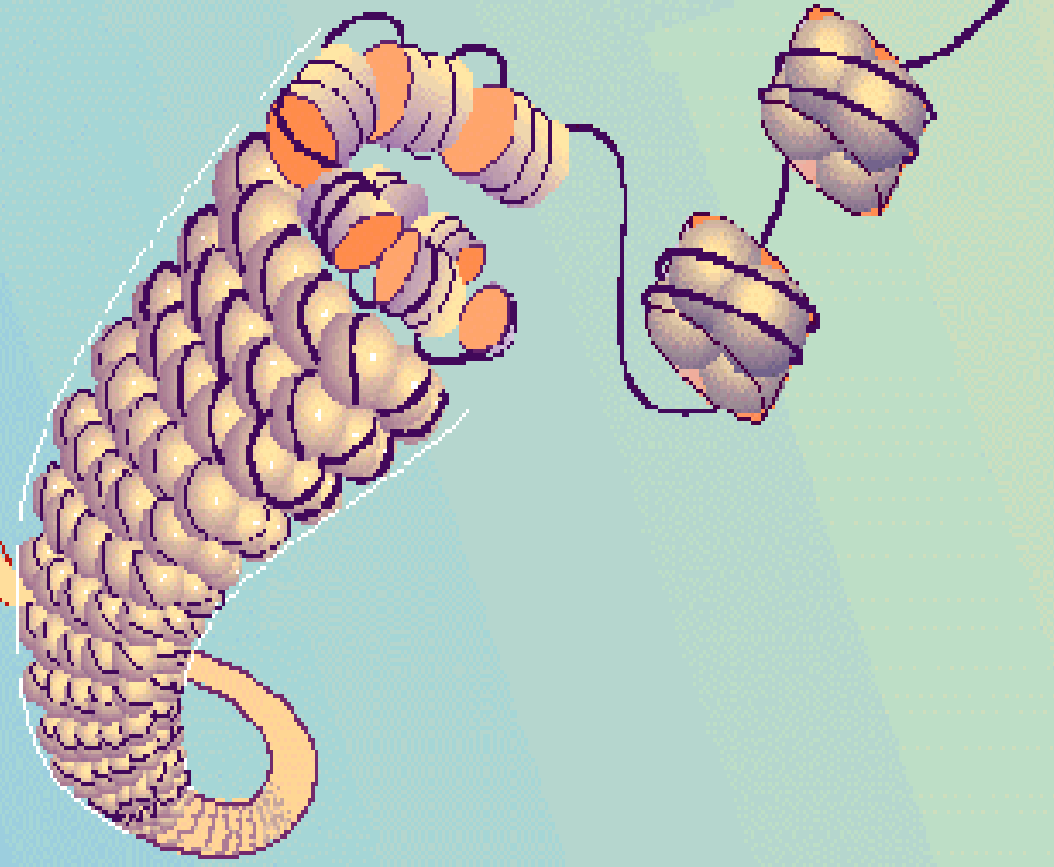
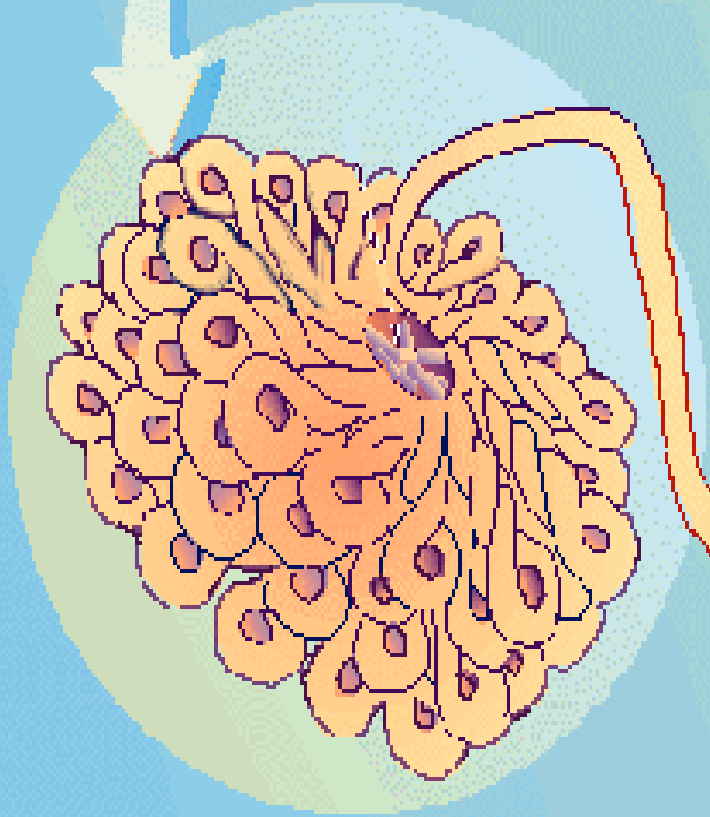
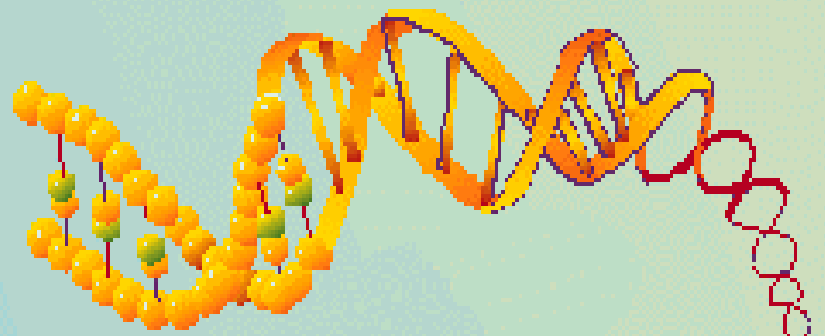
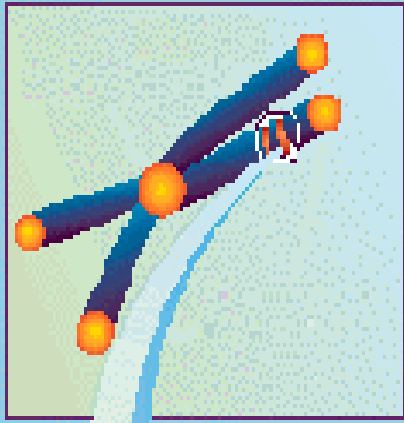


M₁dG in colon DNA

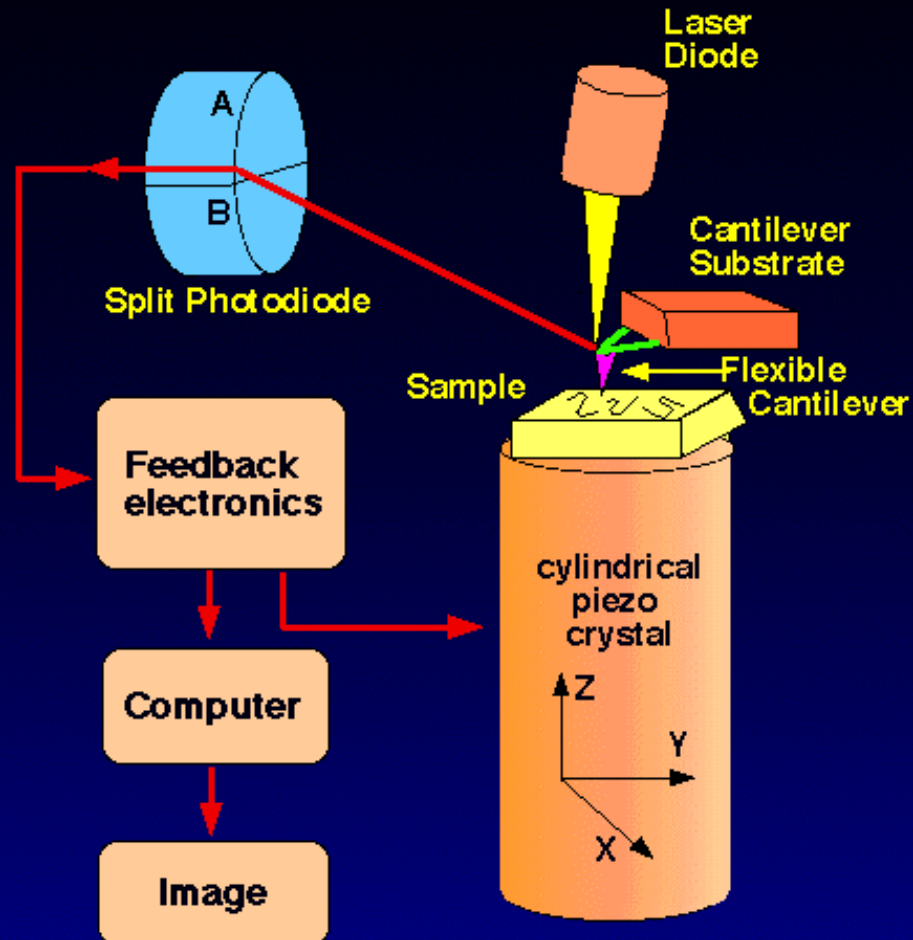


Leuratti *et al.*,
CEBP, 2001

- Most methods for DNA adduct measurement effectively measure average levels of damage across the genome but p53 results suggest that certain types of adduct are likely to be much more important for cancer risk
- Atomic force microscopy (AFM) has the potential to allow physical localisation of specific types of DNA damage within particular sequences



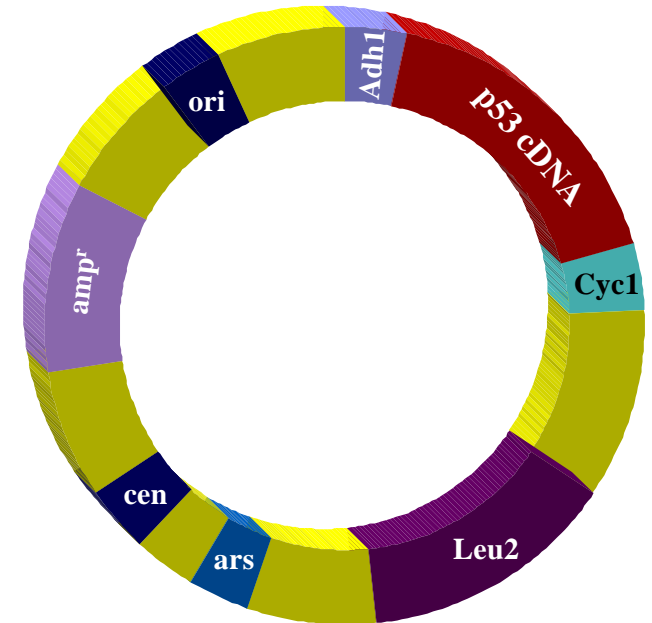
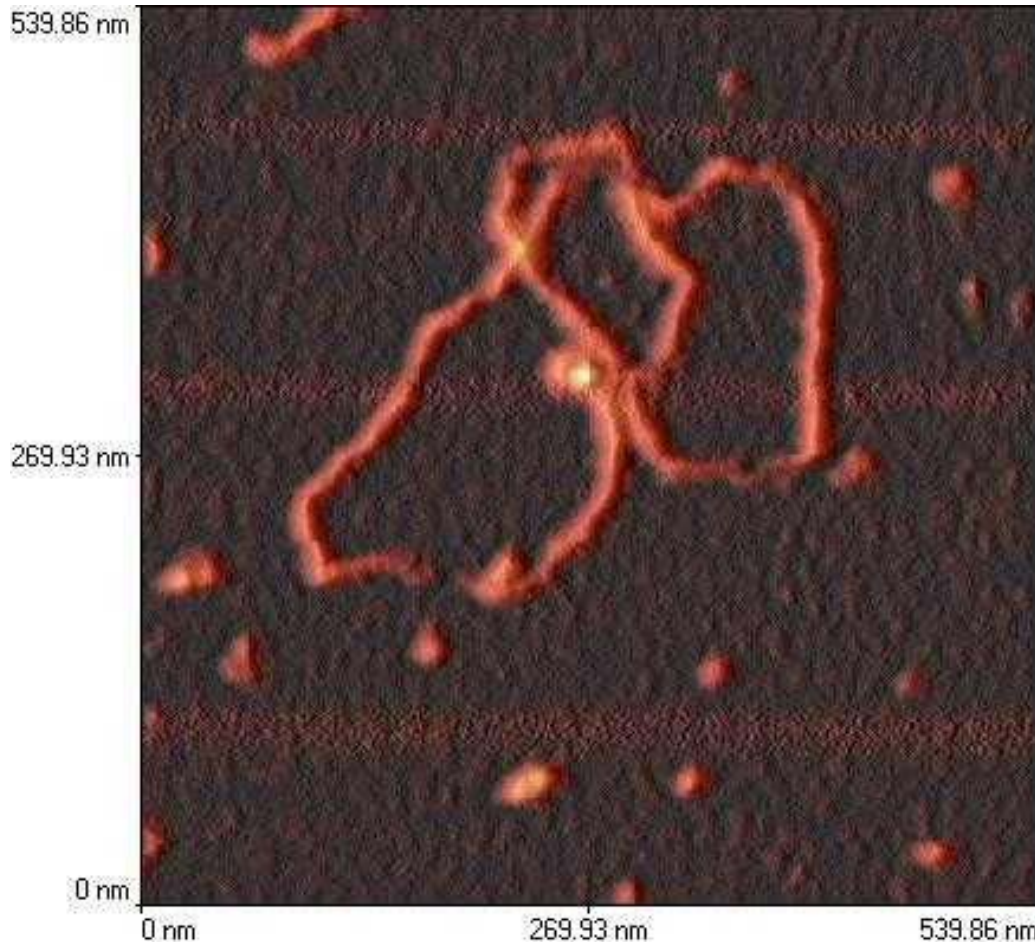
Atomic Force Microscope Sensing System



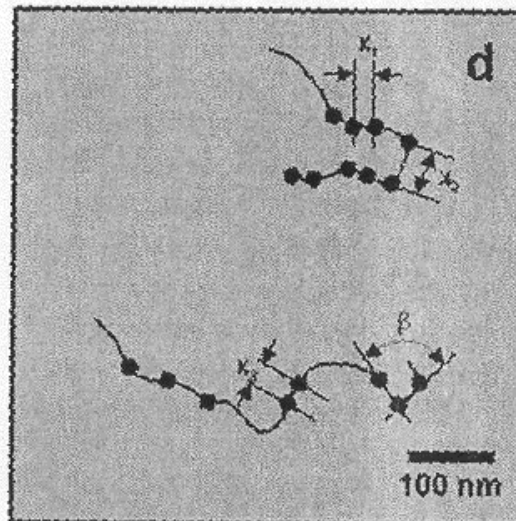
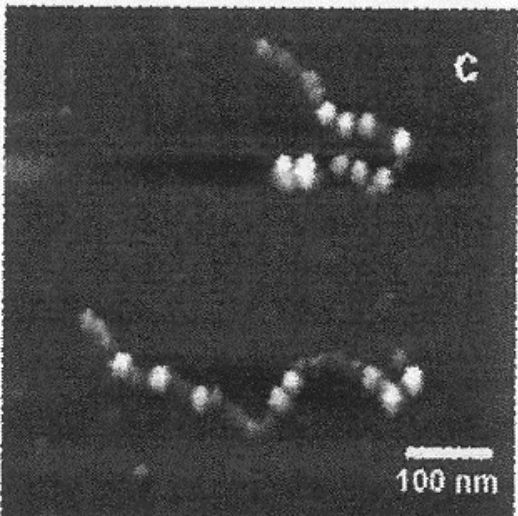
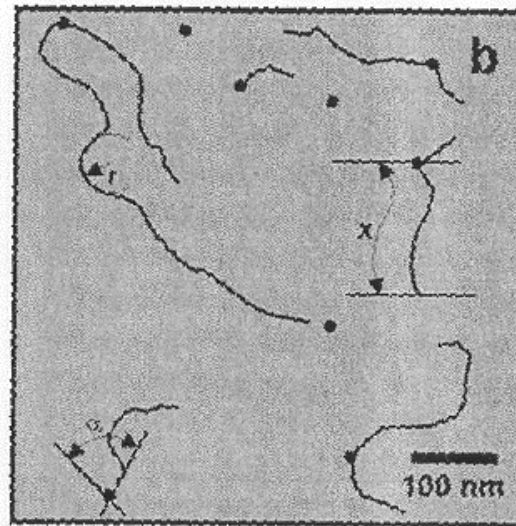
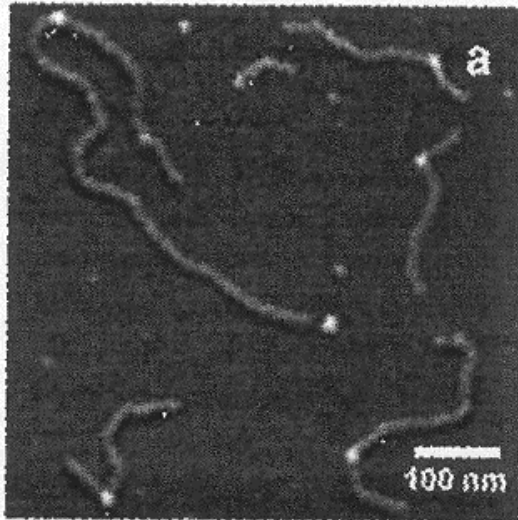
AFM in practice



Visualisation of single DNA molecules



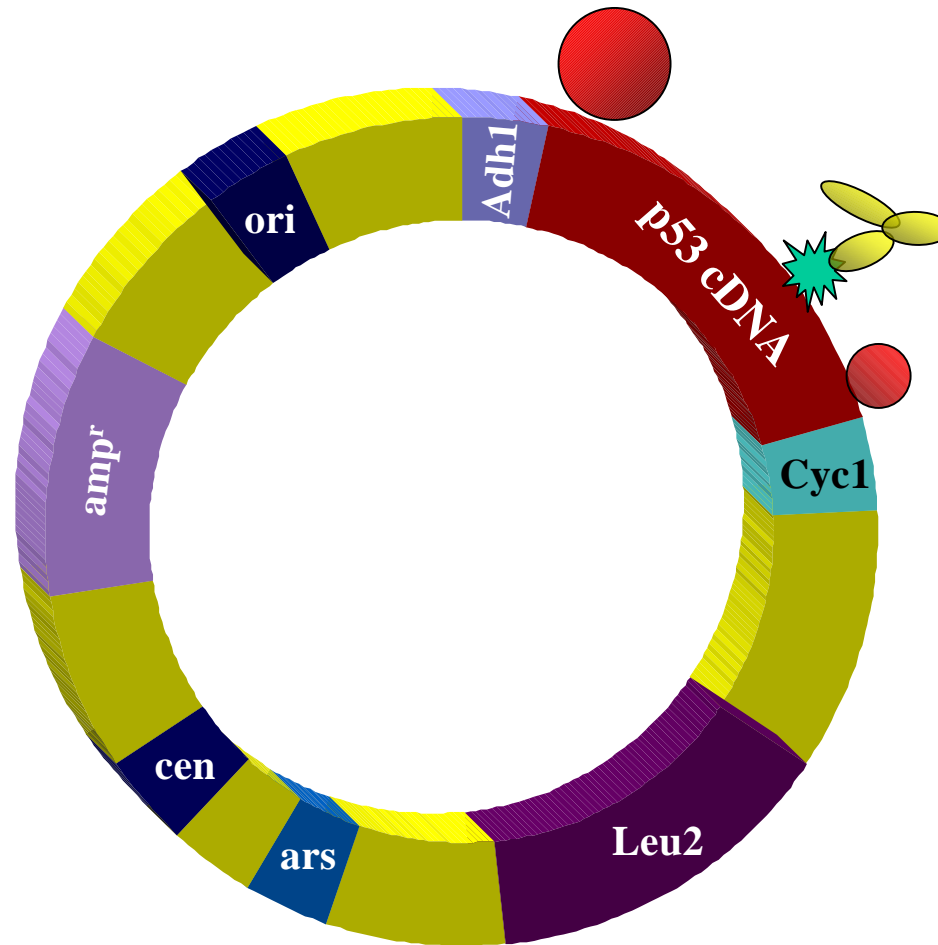
Physical mapping of DNA damage



DNA-protein complexes

Van Noort S.J.T. *et al.*,
Nucl. Acids.Res., 1999

Mapping DNA damage



Summary (1)

- There is good evidence that measurement of DNA damage can be a useful indicator not only of exposure but also of risk of disease
- Numerous methods are available for detection of DNA damage at levels relevant to “real” human exposure
- Overall the major risks for adult human cancers come from subtle interactions of exogenous and endogenous factors for which DNA adducts can be informative quantitative markers

Summary (2)

- However, exposures to known leukaemogenic chemicals (such as benzene and butadiene) are low
- Collecting blood and tissue samples from children to extract DNA or proteins for adduct analysis is difficult for good reasons
- Given the rarity of the outcome it seems unlikely that biomarkers of DNA damage are going to be particularly useful in studies of leukaemia in children

Acknowledgments



- Chiara Leuratti
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(Leeds)



FOOD
STANDARDS
AGENCY