Biodosimetry for radiation-exposed individuals (and for other genotoxics…)

CEA Grenoble
Nucleic Acid Lesions Laboratory

CEA Program Technologies for Health
CEA Program Toxicology

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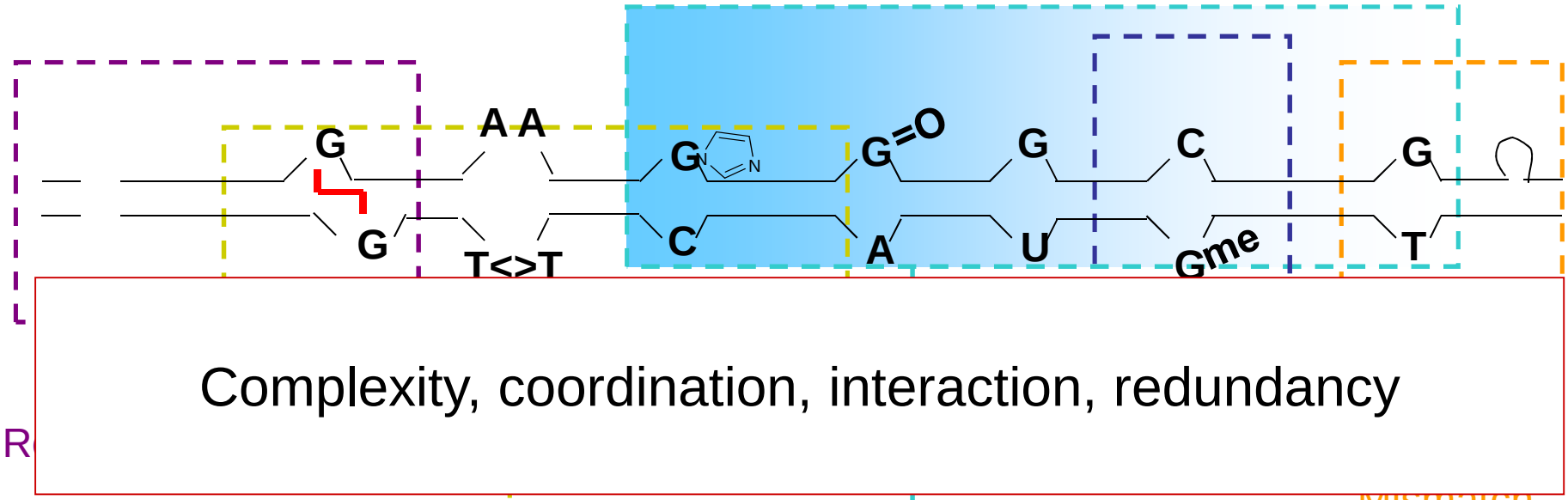
A diversity of DNA lesions – A diversity of DNA repair pathways

Genotoxic agents :

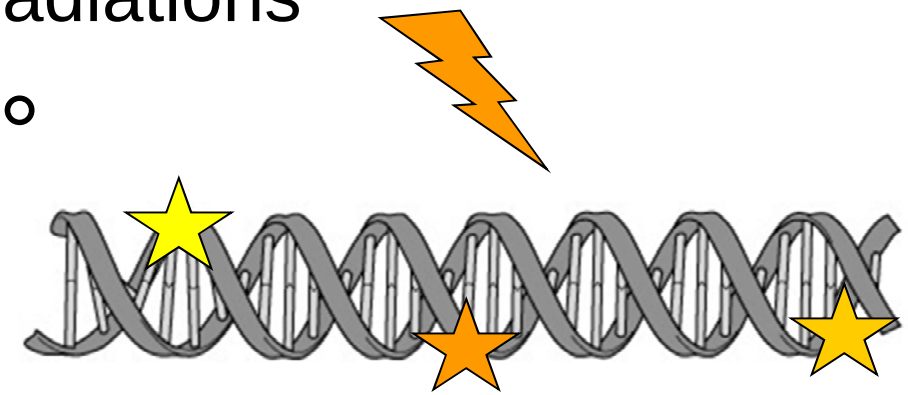
UV, PAHs, oxidative stress, ionizing radiations, drugs, etc



2 pathways share common « cut and paste » mechanisms (Excision/Synthesis Repair)

Ionizing Radiations

OH°



DSB
Oxidized bases

DNA Damage Response **DDR**

Signaling pathways ++
ATM, p53, NF-KB, etc

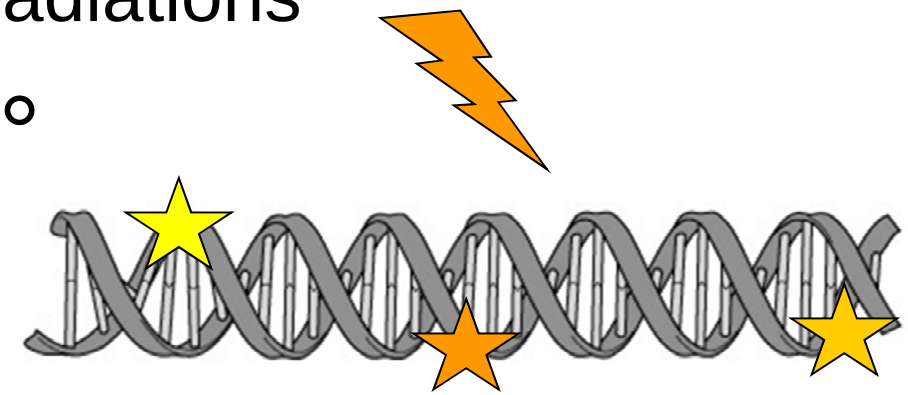
Cell cycle arrest
Apoptosis
Necrosis
Transcription

DNA Repair

ROS (OH°) induced oxidized bases are taken in charge by BER
DSB are taken in charge by recombination mechanisms

Ionizing Radiations

OH°



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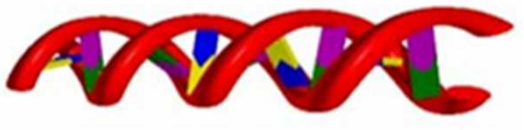
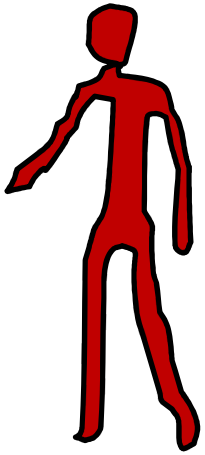
Genotoxic Exposure

DNA Repair



Individual features:

Polymorphisms (“normal” variability)
Hereditary diseases
Life style, age, cumulative exposure



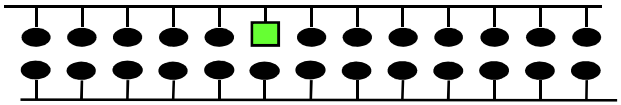
70 % « normal »
20 % « mildly » sensitive
5-10% « hyper » sensitive

Risk of acute effects or
long term seconday effects

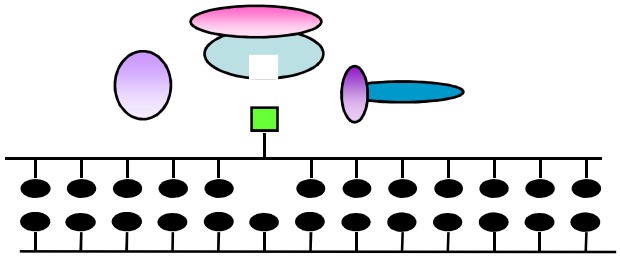
→ Identify to take in charge

Excision/Synthesis Repair Mechanisms

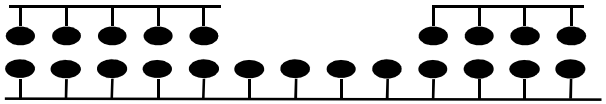
Lesion



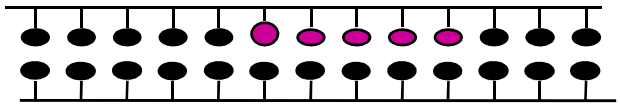
Recognition



Excision
(glycosylases/APE1, NER, ICL)

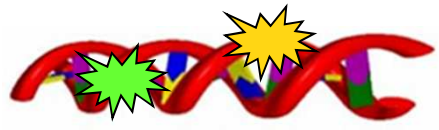


DNA synthesis
(polymerases)



Base Excision Repair
Main actors are Glycosylases and AP Endonuclease

What is the issue ?



induces DNA Repair Adapted response Specific pathways

In the absence of information about the individuals' exposure level :

?

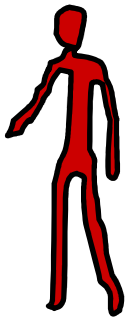
DNA Repair Sensor

Biomarker of exposure (acute/chronic)

Inform on the nature of the genotoxic (MOA)

Dosimetry tool (level of exposure)

Predict associated risk (cancer, inflammation)



To bring some answers

→Establishment of a DNA Repair Signature following radiotherapy regimen (mimics OH° attack)

Relevant way to establish a DNA Repair Signature

DNA Repair is a System

→ Because of post-translational modifications, epigenetic regulation, protein translocation, measurement of repair enzyme activity is more relevant than measurements at genes, transcripts, proteins levels

→ Because of redundancy and complexity, measurement of 1 activity cannot characterize a whole pathway

→ A comprehensive approach is more suitable

Concept	Multiplexed enzymatic repair assays	Quantitative Relative contribution of each subpathway Co-regulations

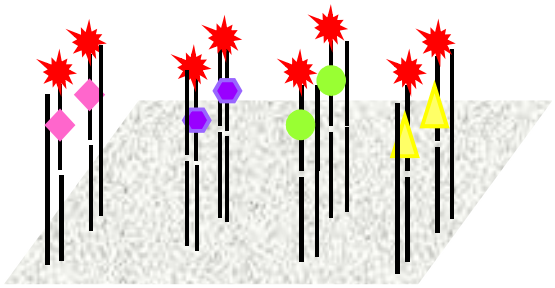
Assay: « ODN cleavage assay »

Relies on the specific cleavage of substrate lesions by glycosylases/AP endonucleases

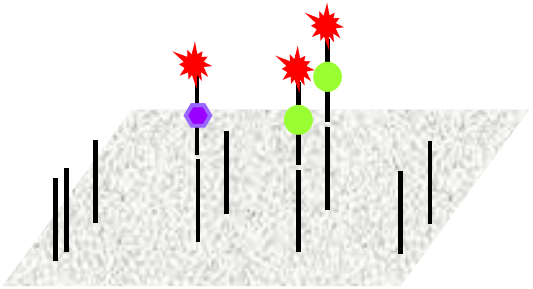
→ multiplexed version on Biochip

Multiplexed Oligonucleotide (ODN) Cleavage Assay

Glycosylases - AP endonucleases Signature

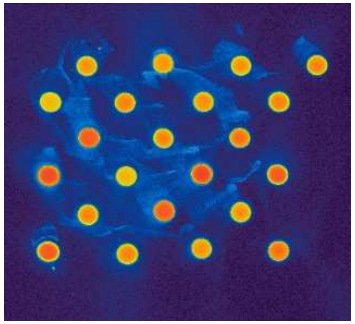


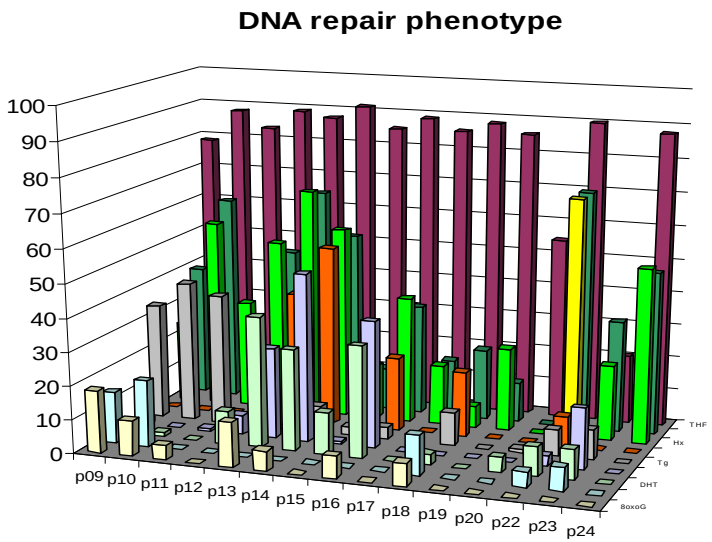
Extract

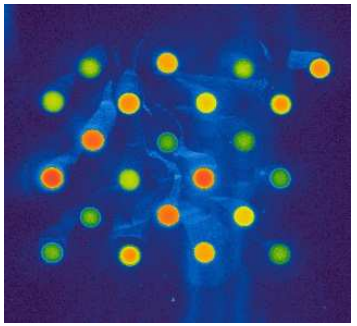


ODN – specific lesions

Fluorescence loss







DNA Repair Signature (Excision –10 lesions → 10 enzymes)

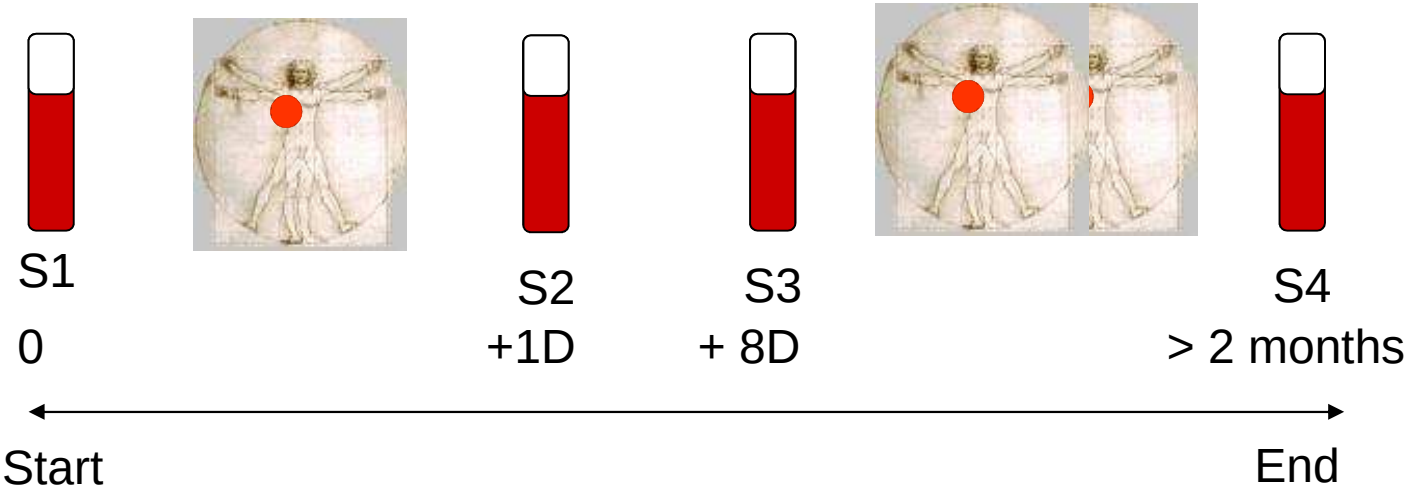
Covers repair of most of lesions induced by OH° (except DSB)

Proof of concept: Effect of Radiotherapy Regimen on DNA Repair Signature

15 patients with various cancer types

OH°

2 Gy



CPT ™ tubes (BD) → PBMCs isolation → Nuclear Extracts → Protein Concentration → Repair Assay

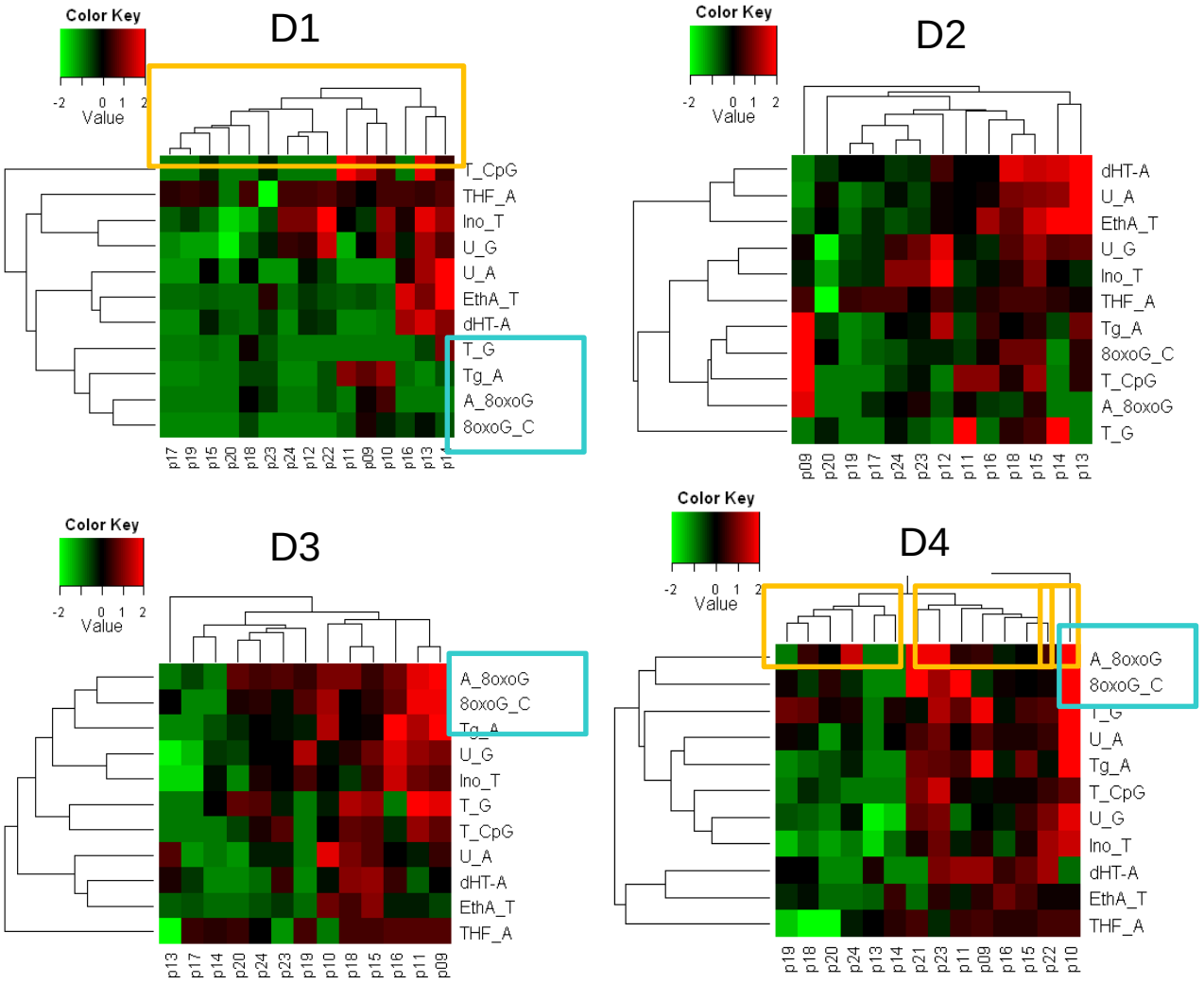
Plate-Array Format

CHU Grenoble, Service *of Pr J. Balosso*.
Blood sampling perfomed under the supervision of *Dr M. Rastkhah*

Data treatment - Results

All data (% of cleavage) centered around 0 (Mean = 0; SD=1)

Classification according to similarities: hierarchical clustering



Impact on Repair

8oxoG_C (hOGG1):
D2 and D3 > D1

A_8oxoG (MUTYH):
D3 and D4 > D1

Tg_A (NTH, NEIL1):
D3 >D1

T_G (MBDH):
D4 > D1

Conclusion

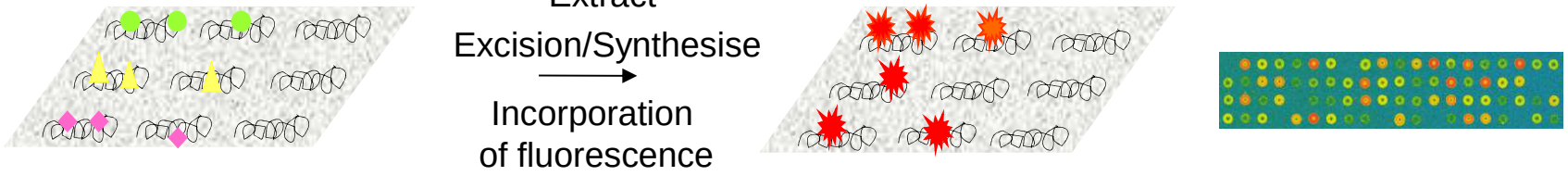
Up-regulation of glycosylases that take in charge oxidized bases
→ Adaptation to the stress (OH°)

DNA Repair Signature = Biomarker of Exposure

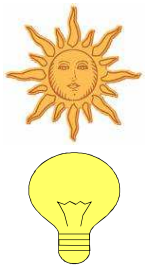
After 4 radiotherapy doses
- 2 subgroups for response profile
- 1 atypical patient → at risk for adverse effect ?

Due to specific molecular features that would be responsible for a particular susceptibility to stress :
Dysfunction/deregulation of DDR ?
Inflammation ?
→ Requires correlation with clinical data

Other data supporting the relevance of the DNA Repair Signature as Biomarker of Exposure



Plasmid Biochip - Excision/Synthesis Repair



- Chronic Sun Exposure (**UV+ROS**) impact **all** repair pathways
- **Chronic Sun Exposure** + **UVB** impacts repair pathway that takes in charge **UVB lesions**

Human normal fibroblasts
Prunier *et al*, Mutat Res, 2012

- **Cisplatin** treatment impacts pathway that repairs **Cisplatin lesions**
- **Drugs** with similar **MoA** display **i**dentical signature

Cancer cell lines
Forestier *et a*l, 2012, Plos One

Conclusion

DNA Repair Sensor

?

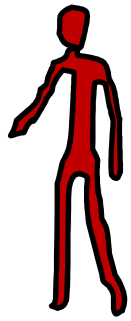
Biomarker of exposure (acute/chronic)

Inform on the nature of the genotoxic

Dosimetry tool (level of exposure)

Predict associated risk (cancer, inflammation)

→ Requires correlation with clinical data



To go further : population study proposal

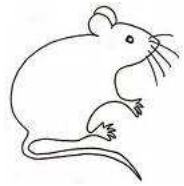
Effet of chemicals, drugs, IR

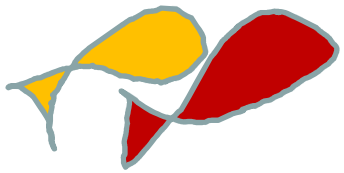
- Get a specific signature, identify specific biomarkers for different types of genotoxics → database
- Investigate dose/response, sensitivity → biodosimetry

Short term response to acute stress

Ex vivo on exposed human blood
Dose/Response (max 24h)

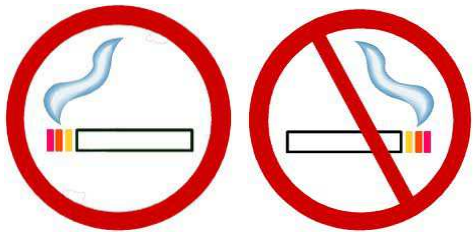
Long term response to chronic exposure



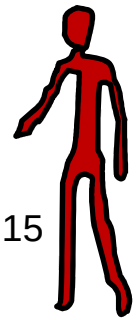


Using animals (rodents, fish)
Dose/response

A possible first approach with volunteers: smokers /vs non smokers



Confounding factor = age



Thierry Douki

Jean-Luc Ravanat

LÉSIONS DES ACIDES NUCLÉIQUES

LXRepair

DNA Repair Signature

Dual applications
Safety/Environnement
- Exposure Biomarkers
- Risk Biomarkers

Oncology
- Personalized Therapy