LRI -EUROTOX- Innovative Science Award

Proposal Title

Evaluation of interactions between PAH exposure and genetic factors on telomere dysfunction in susceptible populations (occupational PAH-exposed subjects and children).

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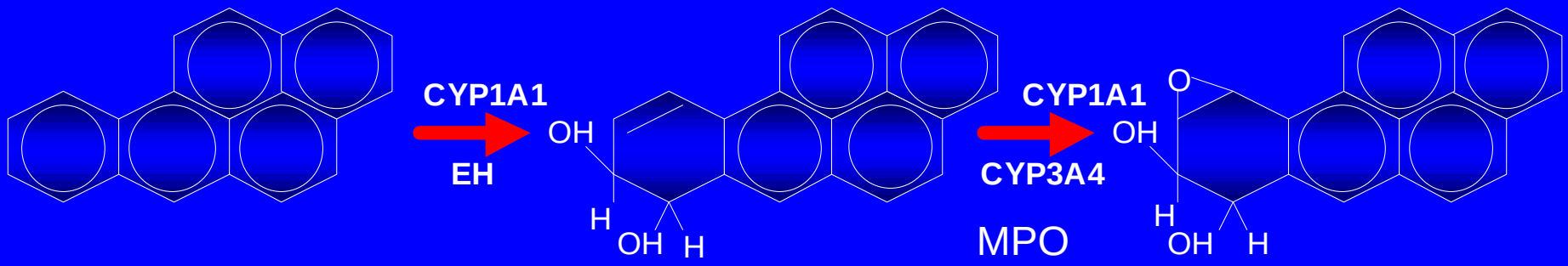
Polycyclic aromatic hydrocarbons (PAHs)
are a widely distributed class of genotoxic carcinogens found in :

- environmental pollution
- diet
- tobacco smoke
- occupational exposures

References:
-IARC (1983) *IARC Monographs on the evaluation of the carcinogenic risk of chemicals in humans,* Polynuclear aromatic compounds, Part.1, Chemical, Environmental and Experimental Data, Lyon
- http://www.chemfinder.com

BaP genotoxic activation

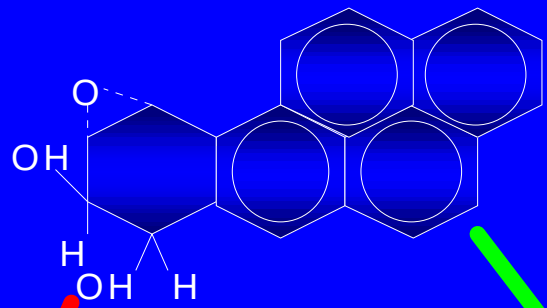


BaP

BaP-7,8-diol

BaP-7,8-diol-9,10-epoxide (BPDE)

Anti-BPDE-DNA adduct is critical in the carcinogenic process of BaP

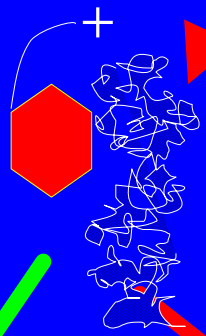


DNA damage

***Anti*-BaP-7,8-diol-9,10-epoxide (BPDE)**

GST (GSTM1)

Non genotoxic coniugates



Point mutations genomic instability telomere dysfunction ?

BPDE-DNA adduct removal by Nucleotide Excision Repair

apoptosis

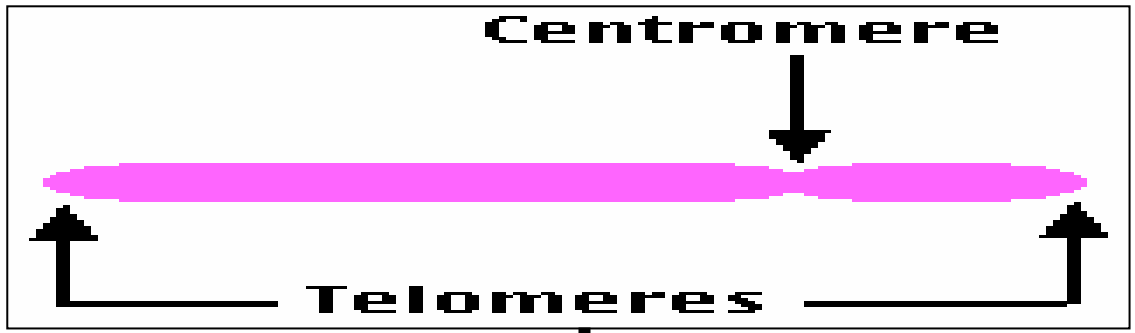
Cancer initiation

Susceptibility factors for anti-BPDE-DNA damage

- Increased activation
- Decreased detoxification
- Suboptimal DNA repair capacity
- Decreased apoptotic capacity

Our hypothesis is that individuals with high levels of damaging anti-BPDE-DNA adducts influenced by susceptibility factors genetically determined will be at high risk to exhibit dysfunction (short) telomeres and genetic instability

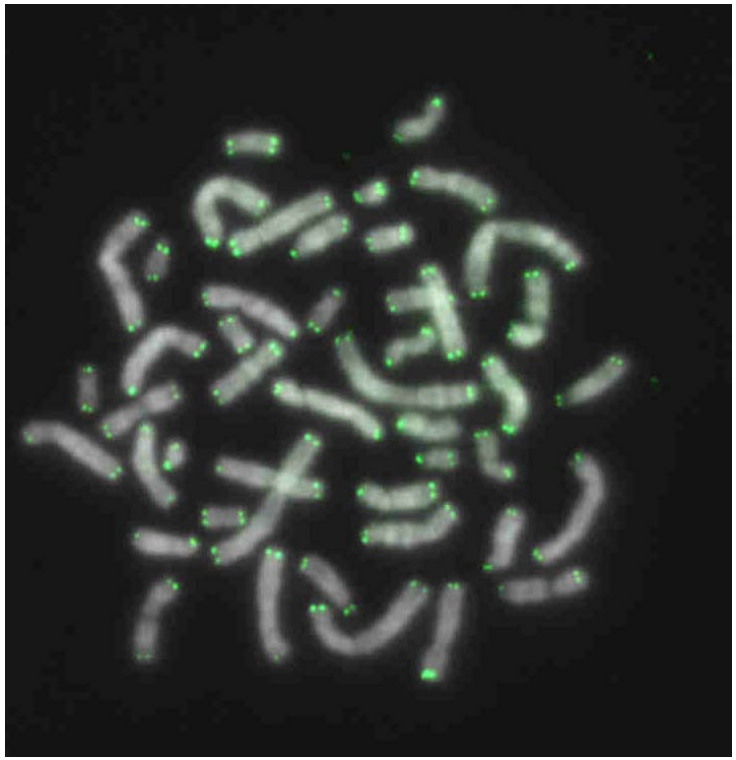
Telomeres are TTAGGG repeat complexes bound by specialized nucleoproteins at the ends of chromosomes in all eukaryotic cells



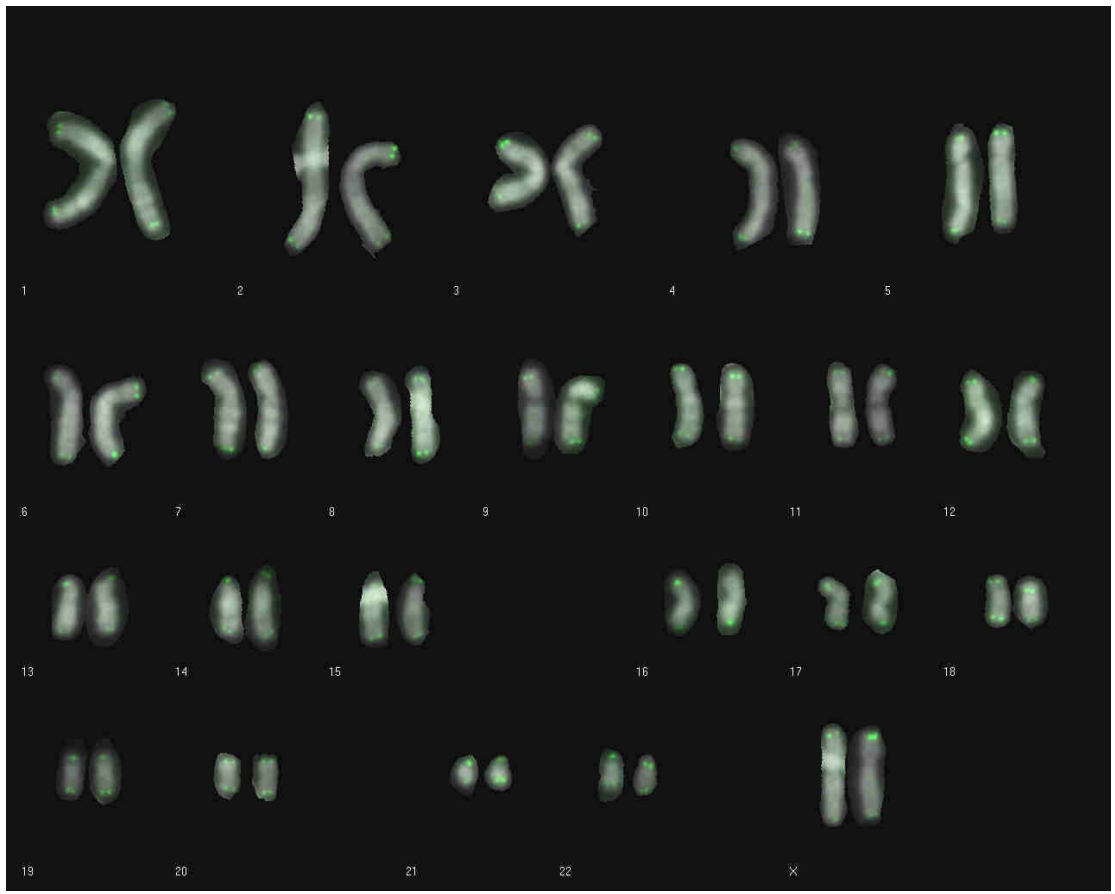
In humans, telomeres consist of as many as 2000 repeats of the sequence 5' **TTAGGG** 3'.

References
Blackburn EH. Cell 2001;106: 661–73.
McEachern MJ, et al.. Annu Rev Genet

Telomeres labelled by a fluorescence peptide-coupled nucleic acid (PNA) probe in Q-FISH



Metaphase of human peripheral blood cells



Telomeres are crucial to the life of the cell

Keep the integrity of the genome

During cell division

By capping the ends of chromosomes prevents:

- •nucleolytic degradation
- •end-to-end fusion
- •irregular recombination

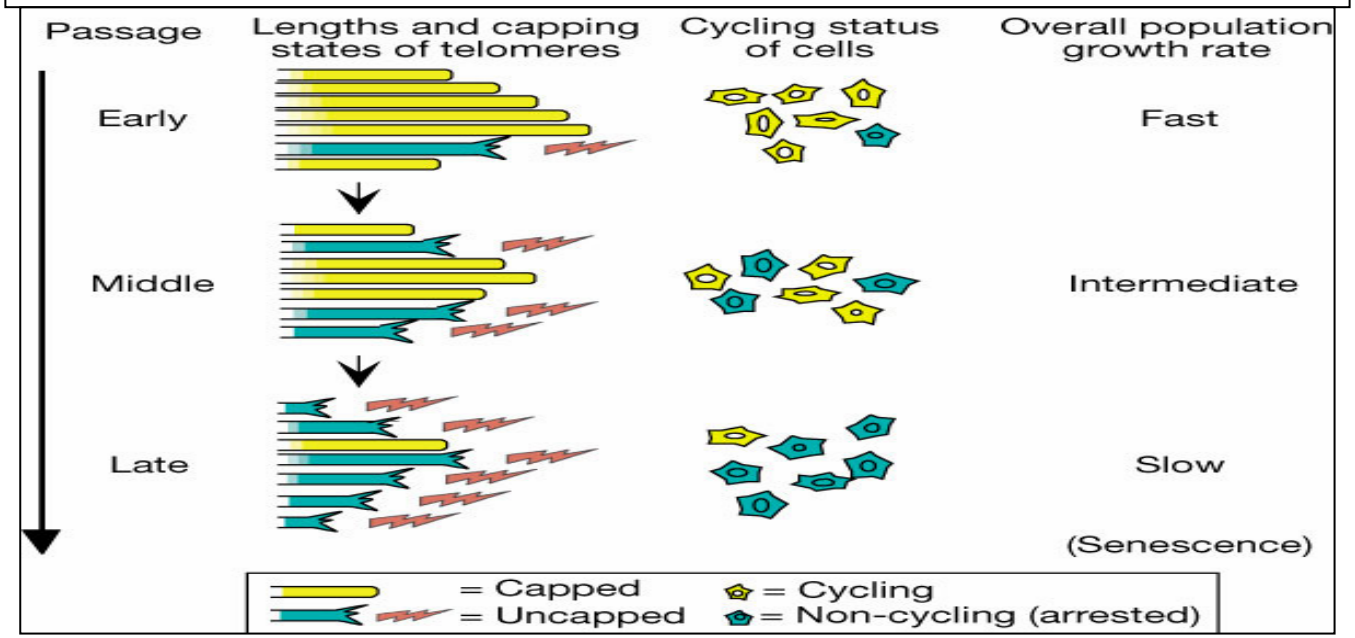
Damage responce

By chromosomal repair :

- •recruits repair enzymes
- • *de novo* synthesis of telomere repeats at double-stranded breaks

This genetic integrity, however, is gradually lost as telomeres progressively shorten with each cell replication cycle.

In normal cells telomere length reduces with aging (20-40 bp/year)



apoptosis

Dysfunction/short Telomeres and Cancer

The possibly derived genetic instability associated with telomere dysfunction/short telomeres is an early event in tumorigenesis

In the neoplastic tissue

- predictor of benign-to-malignant progression
- diagnostic marker for cancer and as a marker for prognosis

In the accessible peripheral blood lymphocytes (PBLs)

- associated with an increased risk for the development of carcinomas of the head and neck, kidney, bladder and lung

Dysfunction/short Telomeres in Hematopoietic Diseases

•In hematopoietic disorders such as chronic myeloid leukemia (CML) and in diseases associated with bone marrow failure such as aplastic anemia (AA), Fanconi anemia and Scwhachmann syndrome (SDS) telomere dysfunction is correlated with disease progression

•The majority of myelodispasia (MDS) show telomere shortening correlated with the clinical behaviour and a linking with apoptosis has been hypothesized.

Dysfunction /short telomere and sensitivity to genotoxic exposure

Correlate with levels of radiation-induced DNA damage or (BPDE) -induced DNA damage in human lymphocytes treated in vitro

Correlate with chromosomal radiosensitivity in lymphocytes from patients with breast cancer and healthy control subjects

ours is the first in-vivo study associating PAH exposure and telomere dysfunction.

Objectives of our ground-breaking work will be the following:

- **Create a Negative (Age) Control Curve of Telomere Length (RTL) using controls of both pedriatic and adult populations**
- **Create a Dose-response Curve of RTL in PAH-exposed adults**
- **Create a Pathologic Curve of RTL in pediatric population**
- **Find an association between telomere dysfunction and anti-BPDE-DNA adduct of adult populations differently exposed to PAHs**

Ultimate goals

1) identify *high-susceptible adult subjects that both for high PAH exposure (detected by high levels of damaging DNA adducts), presence of susceptible factors (metabolic, DNA repair and apoptosis genotypes and/or reduced DNA repair capacity) and telomere dysfunction will be at higher risk of developing cancer*

and

2) identify high susceptible children that due to the telomere length, independent of the pathologic conditions, are at higher risk of developing cancer

Populations

~ 300 Pediatric subjects (birth-17years)

- Pathologic — myelodisplasia-MDS
  myelomonocytic juvenile leukemic (JMML)
  chronic mielomonocytic leukemic (CMML)
- At risk — non clonal dyspoiesis (Anemia Aplastic, Fanconi anemia, Scwhachmann syndrome)
- Controls

~600 Adult subjects (18-60 years)

- occupationally exposed to PAHs
  - -high exposure / coke oven workers
  - -medium exposure/primary aluminium plant
  - -low exposure/urban air pollution (traffic policeman and bus drivers)
- general population in relation to the dietary and smoking behaviours
- controls (non-smoking subjects)

METHODS

	Pediatric	Adults	Subjects with significantly reduced RTL
Blood collection	⇒	⇒	⇒
Hematological Survey	⇒	⇒	
Relative Telomere Length (RTL) Measurement by Flow Cytometry in Peripheral Blood Lympho-monocytes(PBL)	⇒	⇒	
HPLC/fluorescence analysis of anti-BPDE-DNA adducts in PBL		⇒	
Determination of genetic polymorphisms		⇒	
Individual telomere length measurement by quantitative fish (q-fish) in PBL			⇒
Evaluation of spontaneous and FAS inducted apoptosis in PBL			⇒

Integrated Interdisciplinary Project approach

Clinicians

Laboratory of Pediatric Oncohematology
Ambulatory of Preventive Medicine

Epidemiologists

Department of Environmental Medicine & Public Health

Molecular biologists

Department of Environmental Medicine & Public Health

Innovative features:

-Measurement of relative telomere length (RTL) by flow-FISH

-In the long term, can be a new biomarker of cancer risk in individuals occupationally exposed/non-exposed to genotoxic carcinogenic compounds.

-Lack of in-vivo studies associating PAH exposure and telomere dysfunction rendering our results of interest to the most prestigious journals in the field of toxicology, epidemiology and cancer research.

-Interdisciplinary collaboration of clinicians, epidemiologists and molecular biologists to evaluate future relevance and application in cancer research and in other chronic diseases (i.e. arteriosclerosis, ageing).

-Comparison of the RTL and hematologic patterns from peripheral blood smears in the adult populations to detect possible early myelodisplastic features (anisocytosis, pseudo-pelger form, leukocyte degranulation)

Potential Long Term Benefits to the Field of Toxicology

A goal of toxicology, through the validation of biological markers as early indicators of risk, is the identification of individuals, that due to their life behaviour or occupational exposure to carcinogens and/or their genetic traits, who are at risk of developing cancer.

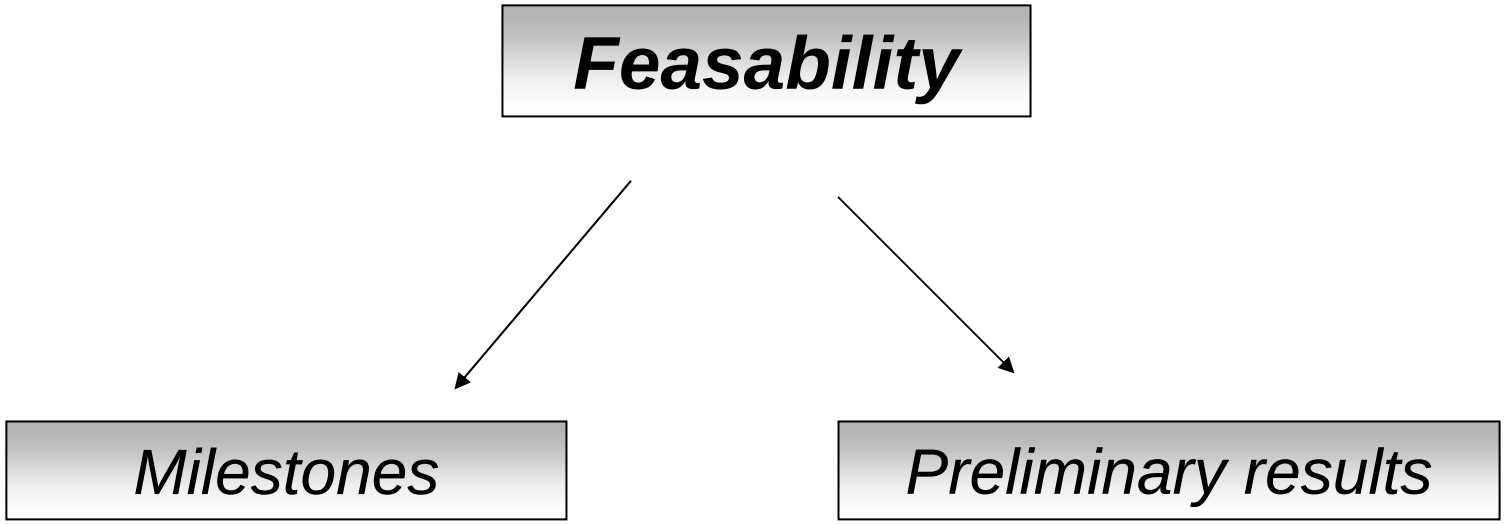
Our study in fact does just this.

•We will analyse various types of at risk populations: those that for high PAH exposure or particular metabolic characteristic are at risk for developing cancer and a RTL evaluation of children with oncologic pathologies and general pathologies.

•We will create a telomere curve for “healthy” individuals with typical environmental exposure, a great benefit for the scientific community as a whole. This in fact has not yet been created in any country.

•This will allow for a reference mark for RTL in highly exposed or pathologic individuals in all areas of scientific research. Additionally, this will allow us to be in the best condition for the validation of biomarkers.

•Functional biological markers of environmental exposures are important in epidemiological studies of disease risk. The study design allows for us to determine whether RTL, an indicator of disease, can be related to the carcinogen exposure


Feasability
Milestones
Preliminary results

Milestones

	First Year	Second Year
Task 1 Sample collection	⇒	⇒
Task 2 Hematological Survey	⇒	⇒
Task 3 Relative Telomere Length (RTL) Measurement by Flow Cytometry in Peripheral Blood (PB) Lympho-monocytes	⇒	⇒
Task 4 HPLC/fluorescence analysis of anti-BPDE-DNA adducts	⇒	⇒
Task 5 Determination of genetic polymorphisms	⇒	⇒
Task 6 A Individual telomere length measurement by quantitative fish (q-fish) in peripheral blood (pb)		⇒
Task 6B Evaluation of spontaneous and FAS inducted apoptosis		⇒
Task 7 Statistical analysis		⇒
Task 8 Evaluation of High Exposure Groups		⇒
Task 9 Data Evaluation		⇒
Task 10 Deliverables/Dissemination Activities		
Yearly Report	⇒	⇒
Posters/Oral Presentations at Congresses	⇒	⇒
Publications in International Journals*		⇒
Seminars on results and innovation	⇒	⇒

Our preliminary results reinforce our belief

that

individuals with high levels of damaging DNA adducts, influenced by metabolic, DNA repair genotype and apoptotic conditions genetically determined, and telomere dysfunction (short telomeres) may be at higher risk for developing cancer and more likely to exhibit genetic instability.

Anti-BPDE-DNA adduct levels in lympho-monocytes of coke oven workers in relation to their *GSTM1* genotypes

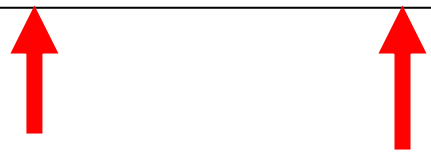
		Coke oven workers	PAH exposure 1-Pyrenol µmol/mol creatinine		*Anti*-BPDE-DNA (adducts/10^8 nucleotides)	
		% (N)	Range	X±SD	Range [a]	X±SD
GSTM1	**active**	67(64)	0.46-31.4	7.06±6.83	1-10.7	3.25±2.01
	****0/*0***	33(31)	0.25-31.1	6.67±8.00	1.04-27.4	5.90±5.59**
	all	100(95)	0.25-31.4	6.93±7.20	1-27.4	4.10±3.78

Coke oven workers with *GSTM1* null (absence of activity) have double the levels of anti-BPDE-DNA adducts

Pavanello et al., Mutation Res. 2004

Linear multiple regression analysis of influence of occupational PAH exposure, *GSTM1*, smoking habits and diet on *anti*-BPDE-DNA adduct levels (n=95) in LMF of coke oven workers.

	PAH exposure	*GSTM1*	Smoking habits	Diet
b	0.159	-2.639	0.176	0.442
SE(b)	0.052	0.751	0.727	0.774
t	3.087	3.512	0.242	0.571
P	=0.0027	<0.001	0.809	0.569



GSTM1 null genotype appears to be as important as occupational exposure to PAHs.

This fact may be the reason for the higher risk of lung cancer in coke oven workers with *GSTM1* null genotype, and suggests that the *GSTM1* genotype could be used as part of routine health surveillance in occupational exposure to PAHs.

52 MDS/Acute Leukemia (AL) patients and controls (ages 5 days-20 years) were analysed for telomere length.

Analysis was done using flow FISH allowed us to identify the average RTL of the groups studied (i.e. controls, non clonal dyspoiesis and MDS/AL patients).

Sainati et al., 2004 in preparation

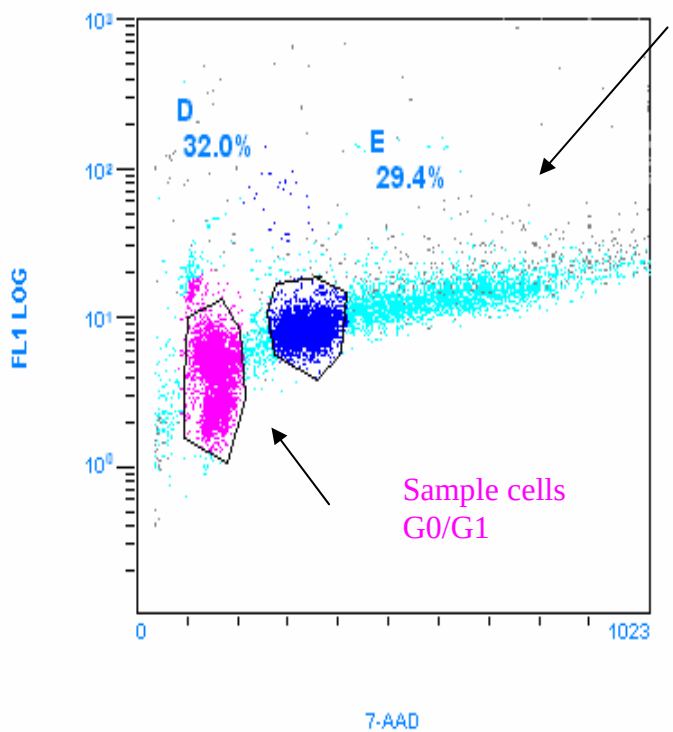
RTL calculation

- (mean Fluorescence of sample with probe – mean Fluorescence of sample without probe) x DNA INDEX of cellular line

RTL= ——————————————————————————— **x 100**

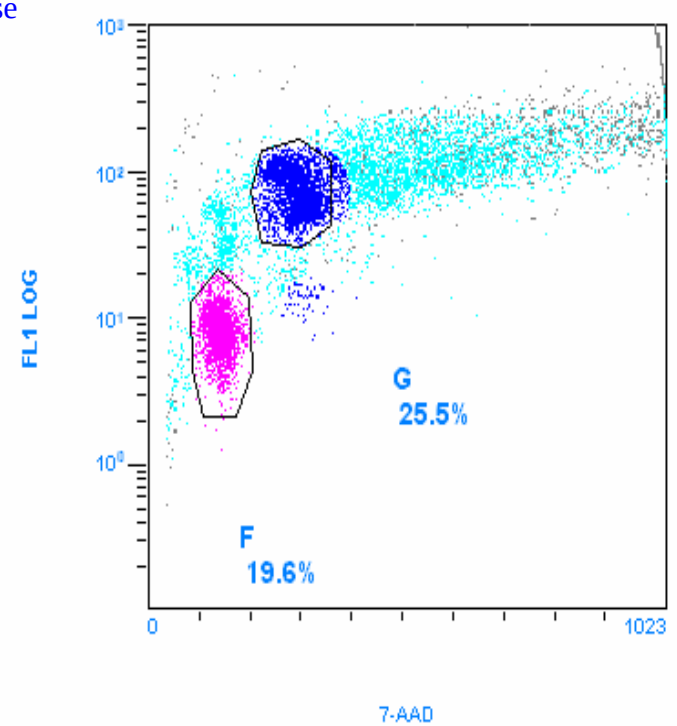
- (mean Fluorescence of cellular line with probe – mean Fluorescence of cellular line without probe) x DNA INDEX of sample

Sample without probe

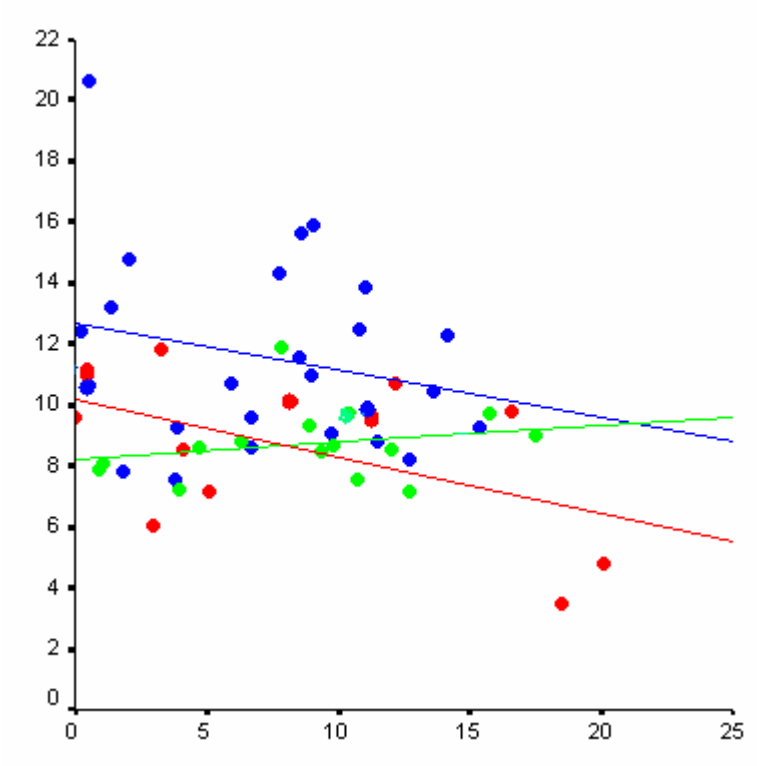


Cellular line 1301
in G0/G1 phase

Sample with probe



Telomere length in 52 MDS/Acute Leukemia (AL) patients and controls



mean RTL

Age

Controls

Non clonal dyspoiesis

MDS/AL

Preliminary Results on relative telomere length

The relative telomere length (*RTL)*average was considerably higher in the control group compared to that of the MDS/AL patients

The average RTL is correlated with age, with an increased rate of shortening of the MDS group compared to the controls.