In vitro evaluation of the genotoxicity of polymeric nanoparticles as carriers for oral drug administration

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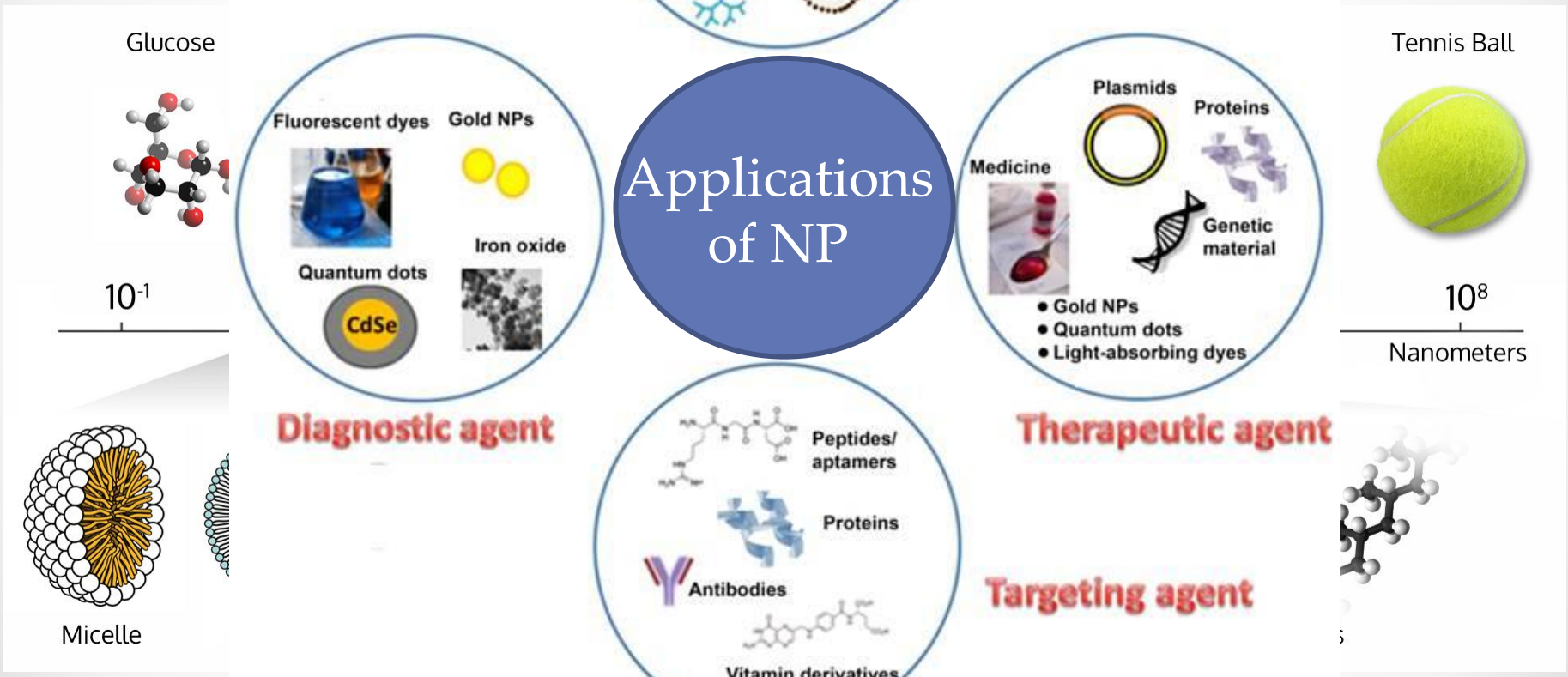
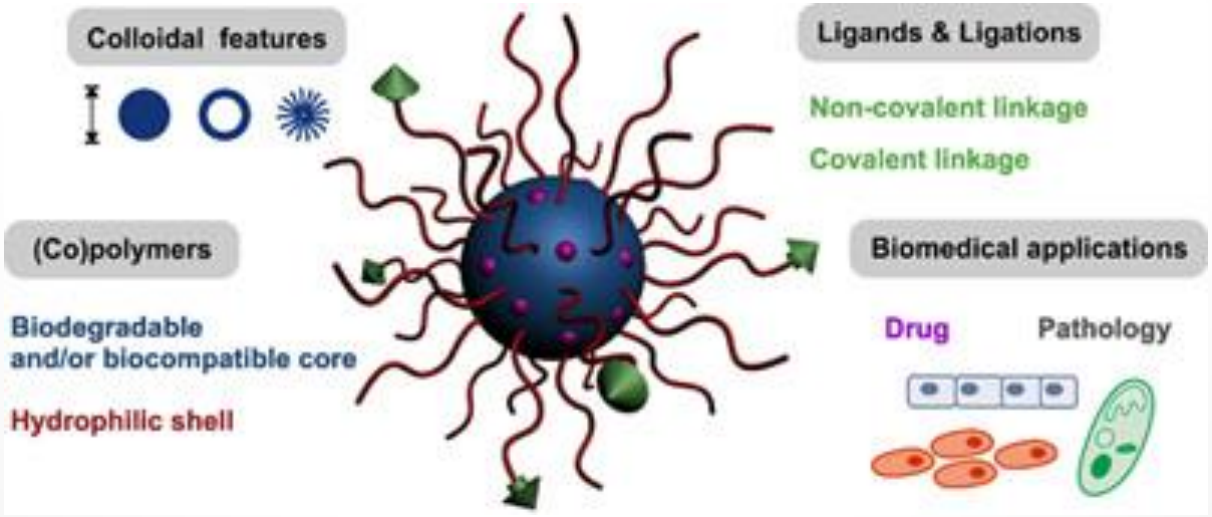
- **Nanop** … nm Ø)


Liposomes
Polymeric NPs
Carrier
Dendrimers
Micelles
Glucose
Tennis Ball
Fluorescent dyes
Gold NPs
Iron oxide
Quantum dots
CdSe
Diagnostic agent
Applications of NP
Plasmids
Proteins
Medicine
Genetic material
• Gold NPs
• Quantum dots
• Light-absorbing dyes
Therapeutic agent
10^{-1}
10^{8}
Nanometers
Micelle
Peptides/ aptamers
Proteins
Antibodies
Vitamin derivatives
Targeting agent

Polymeric NPs

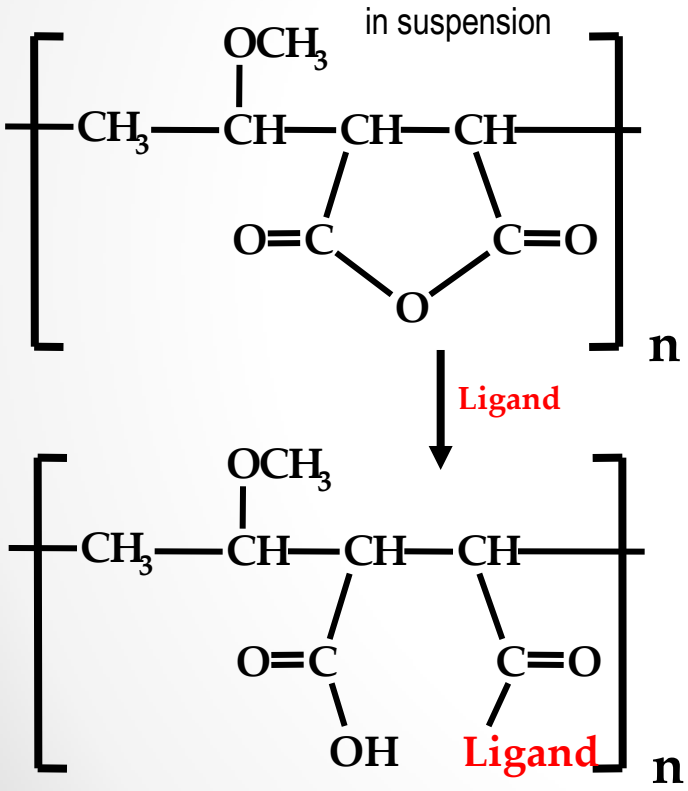
ADVANTAGES

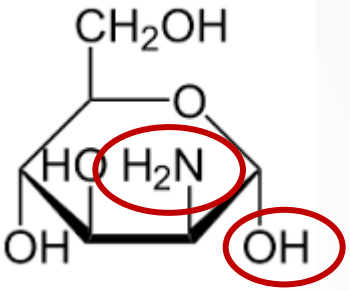
- Promising platforms for drug delivery
- Biodegradables / biocompatible
- Surface modifiable
- Strong bioadhesive interactions with components of the gut mucosa



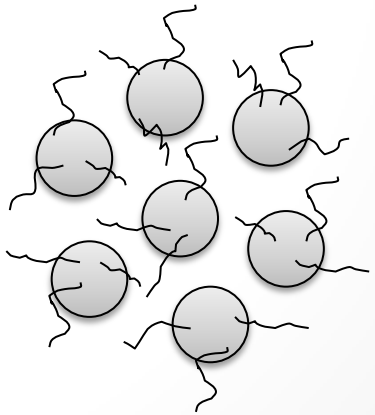
Poly(anhydride) NPs

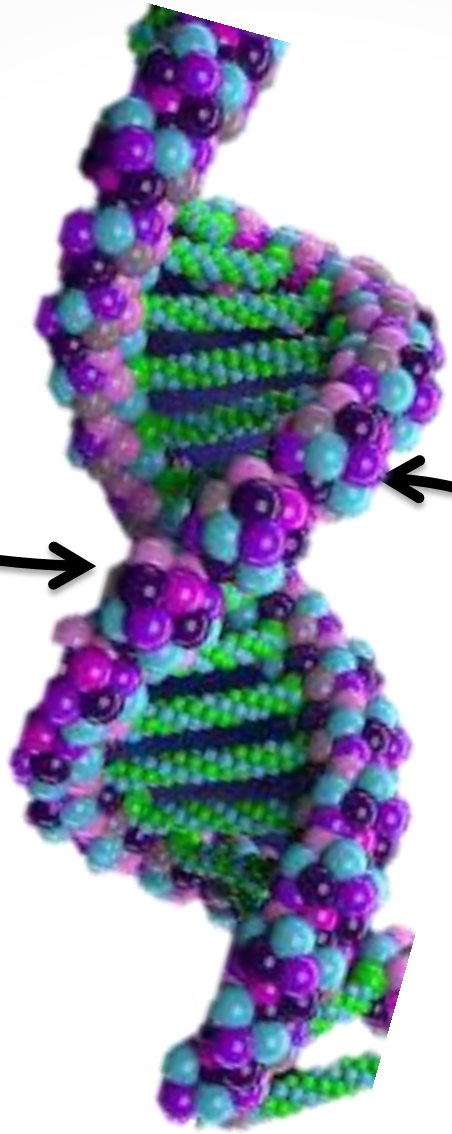
Copolymer of methyl vinyl ether and maleic anhydride (**Gantrez**)





Ligand-Mannosamine





NP

NP

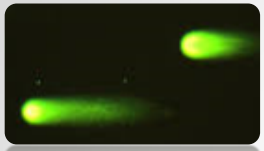
ROS

Direct Damage

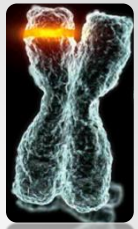
Indirect Damage

AIM

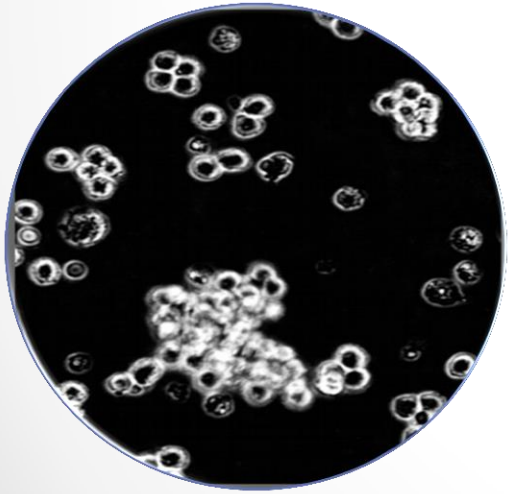
- Evaluate the genotoxicity of two poly(anhydride) NPs, Gantrez and Gantrez-covered with mannosamine.
  - Comet assay in combination with FPG
  - Mouse Lymphoma assay (OECD 490)



Cell line: L5178Y TK$^{+/-}$



DBA/2



- Stable spontaneous MF
- Grow in suspension
- Heterozygous at the TK locus
- Short doubling time

Comet assay

L5178Y TK+/-

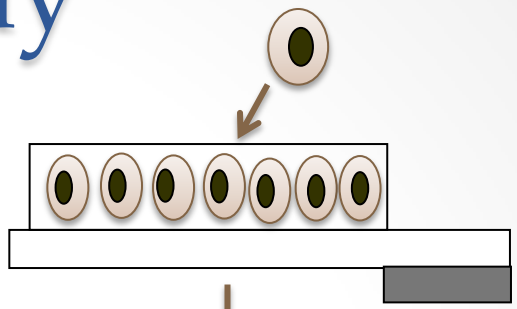
5 [ ] **(mg/mL)**

- 0.0074
- 0.0022
- 0.0670
- 0.2
- 0.6

24 h

C- & C+

N=3

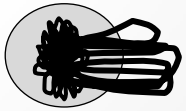


Overnight lysis

Digested with FPG

Oxidized bases

Alcaline unwinding, electrophoresis

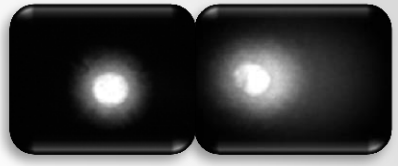


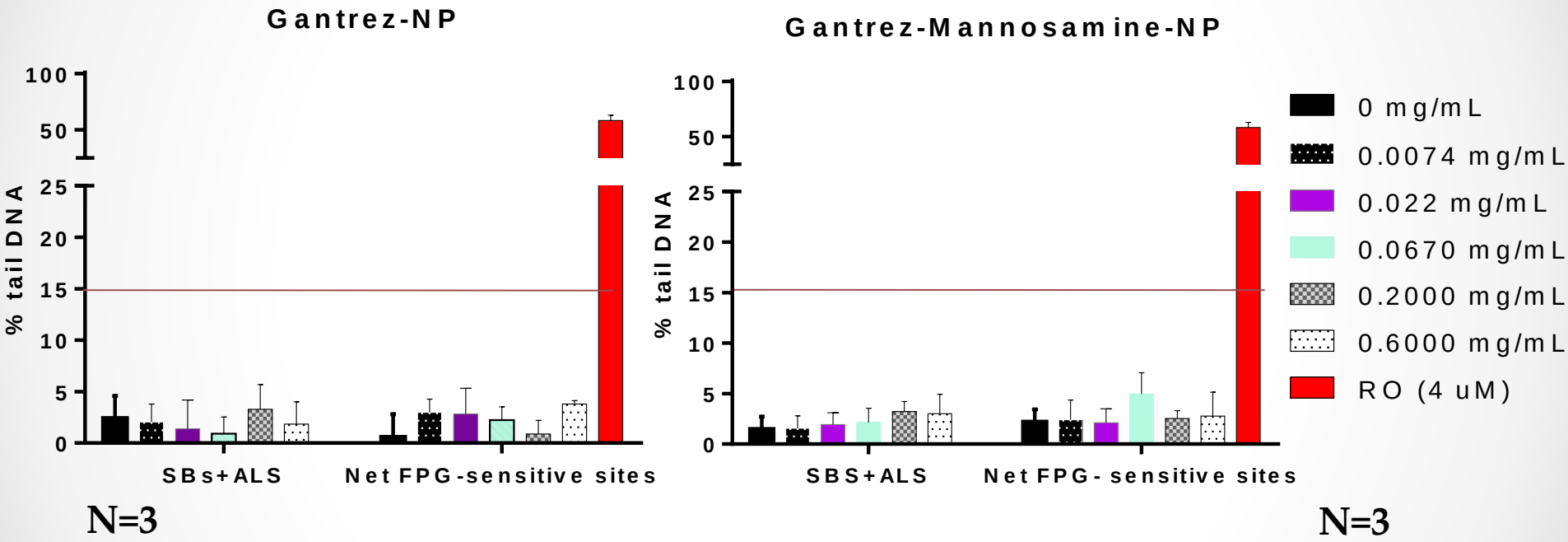
100 comets/slide

Neutralization, staining and visualization

Results

Comet assay





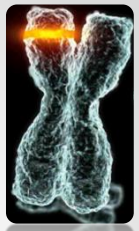
N=3

N=3

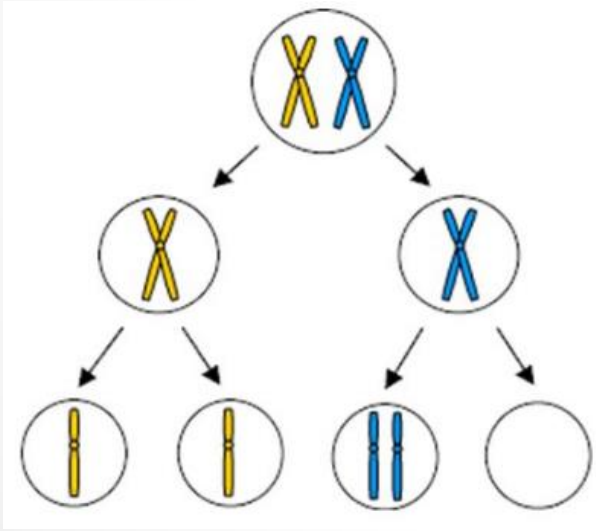
Proliferation assay

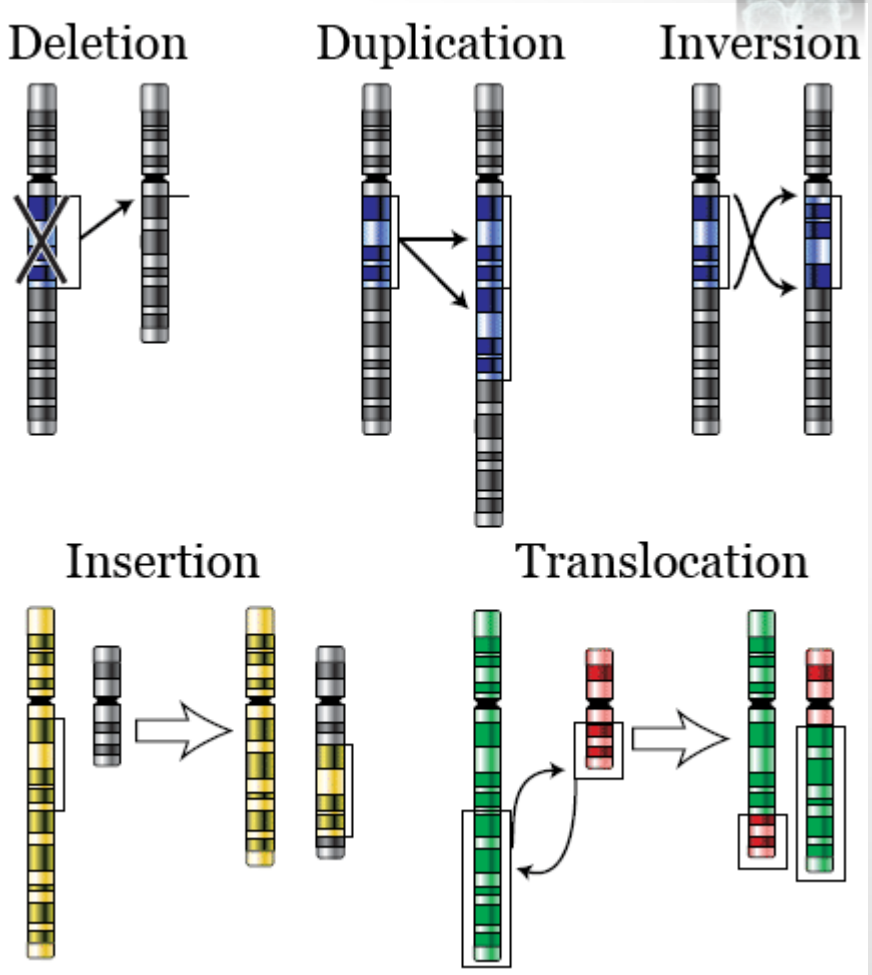
Non-Genotoxic

Mouse Lymphoma assay

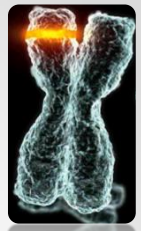


OECD Nº 490 *In vitro* Mammalian Cell Gen Mutation tests using Thymidine Kinase Gene.





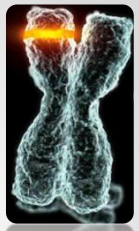
Mouse Lymphoma assay



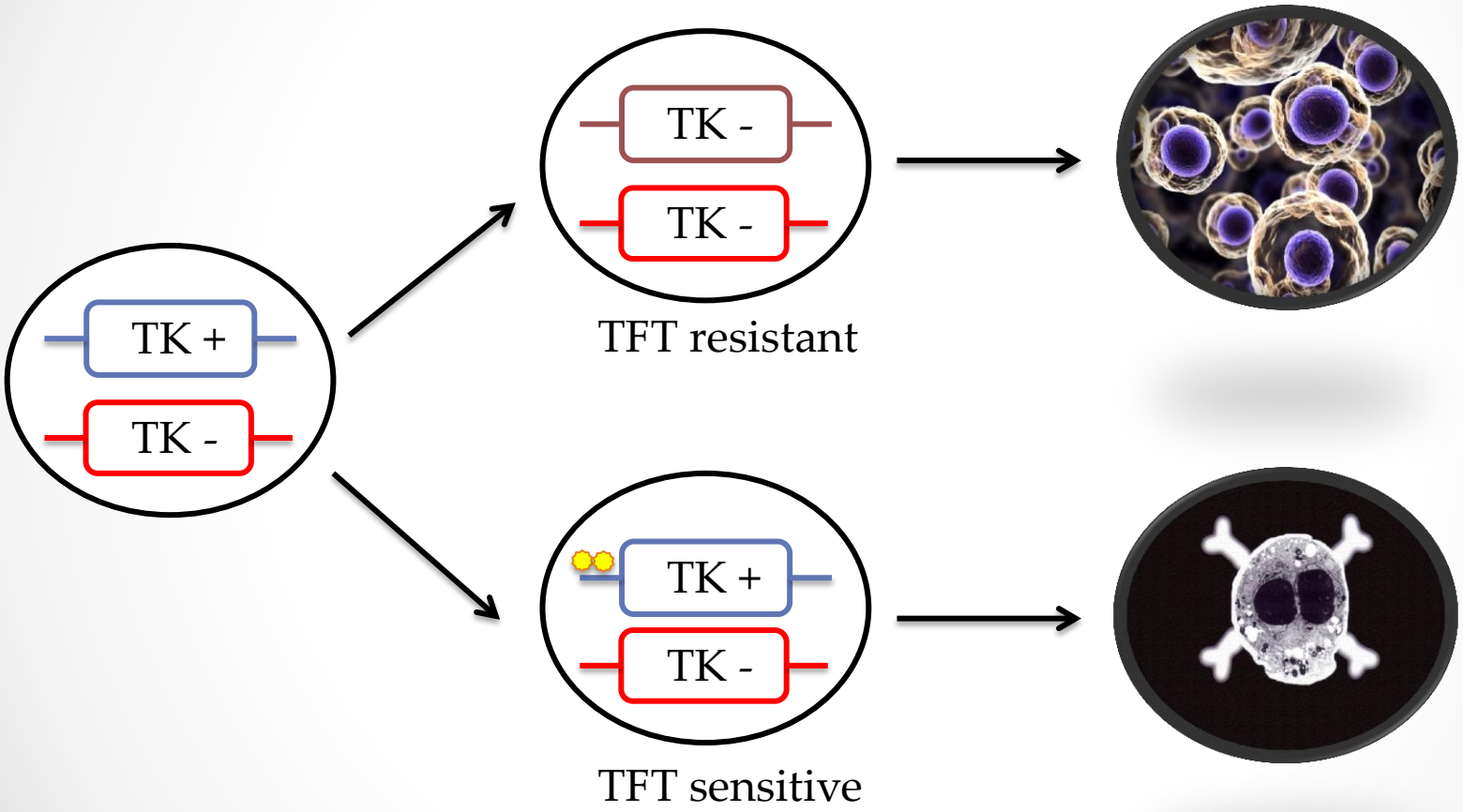
Thymidine $\xrightarrow[\text{Thymidine kinase}]{P}$ Thymidine monophosphate

TFT $\xrightarrow{P}$ Toxic metabolites

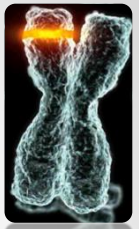
Mouse Lymphoma assay

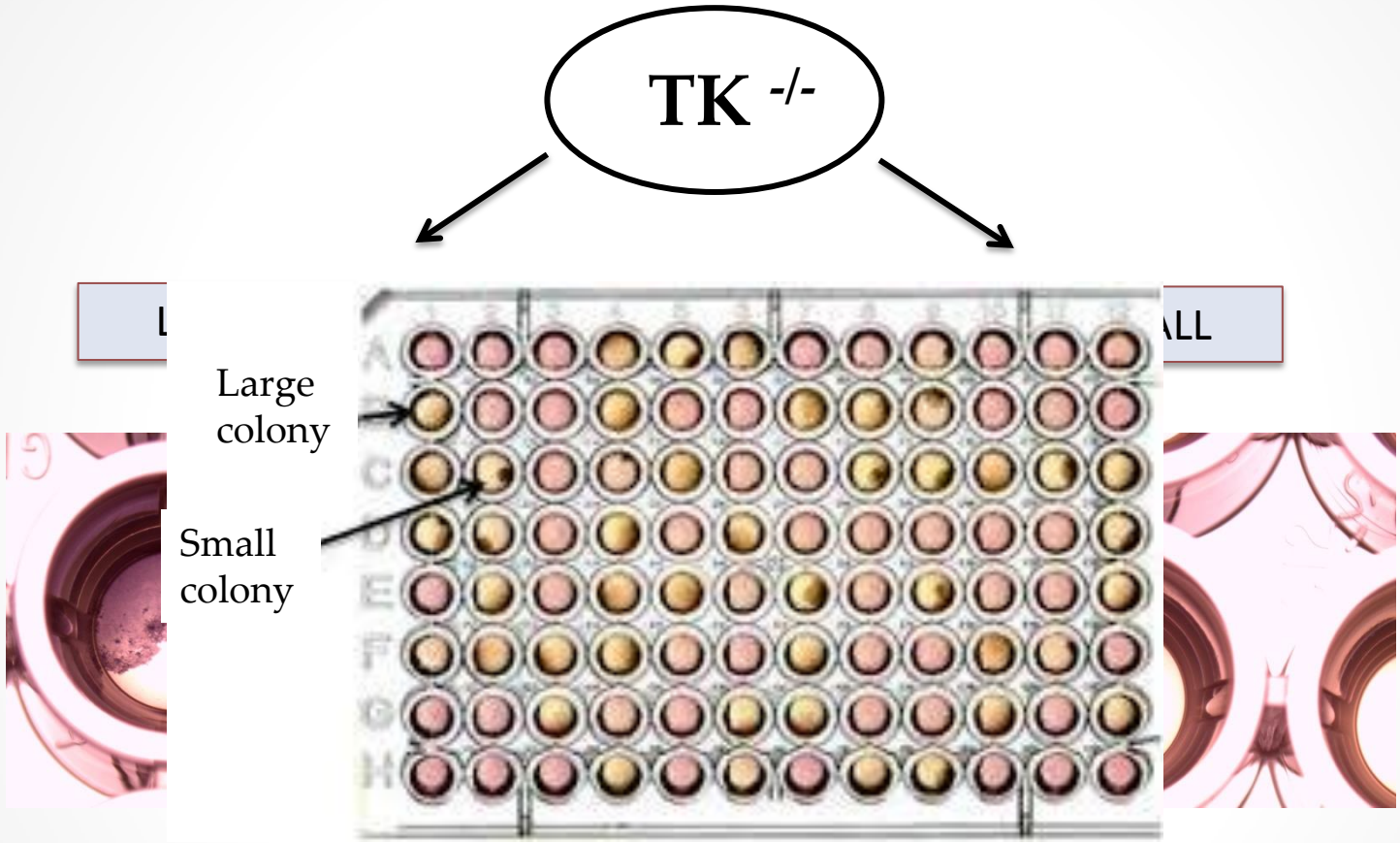


Exposure and TFT selection



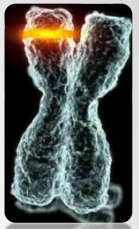
Types of mutants colonies





Events within the gene : base-pair substitutions or deletions

chromosomal aberrations (suggesting clastogenic activity).



24 h of treatment

10[ ]

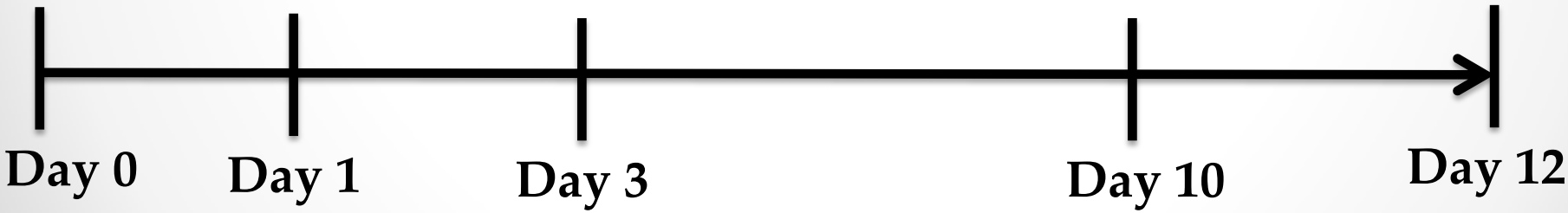
- $3.1x10^{-5}$ mg/mL
- 0.6 mg/mL

C-

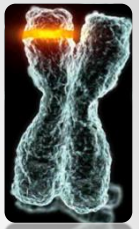
C+

100µM MMS

Treatment period



Assay procedure



Plating Survival

Treatment period

Expression period

Plating Viability & TFT resistance

Day 0 Day 1 Day 3 Day 10 Day 12

Score Survival

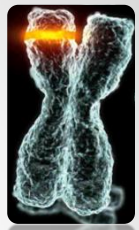
Score Viability & TFT resistance

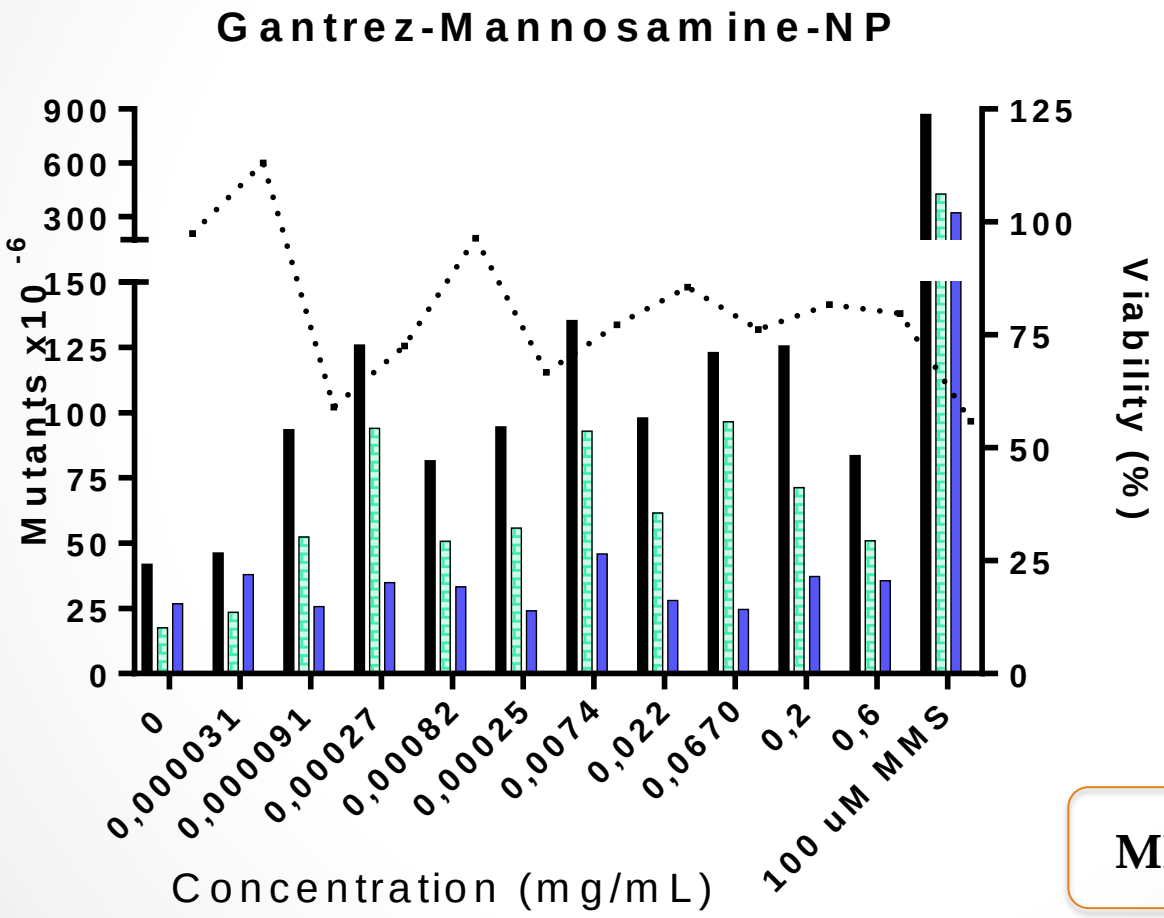
MLA

$$MF_{SAMPLE} \geq MF_{NC} + GEF$$

Results

MLA





$$MF_{SAMPLE} \geq MF_{NC} + GEF$$

Acknowledgment

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Norwegian Institute for Air Research

Roche

Universidad de Navarra

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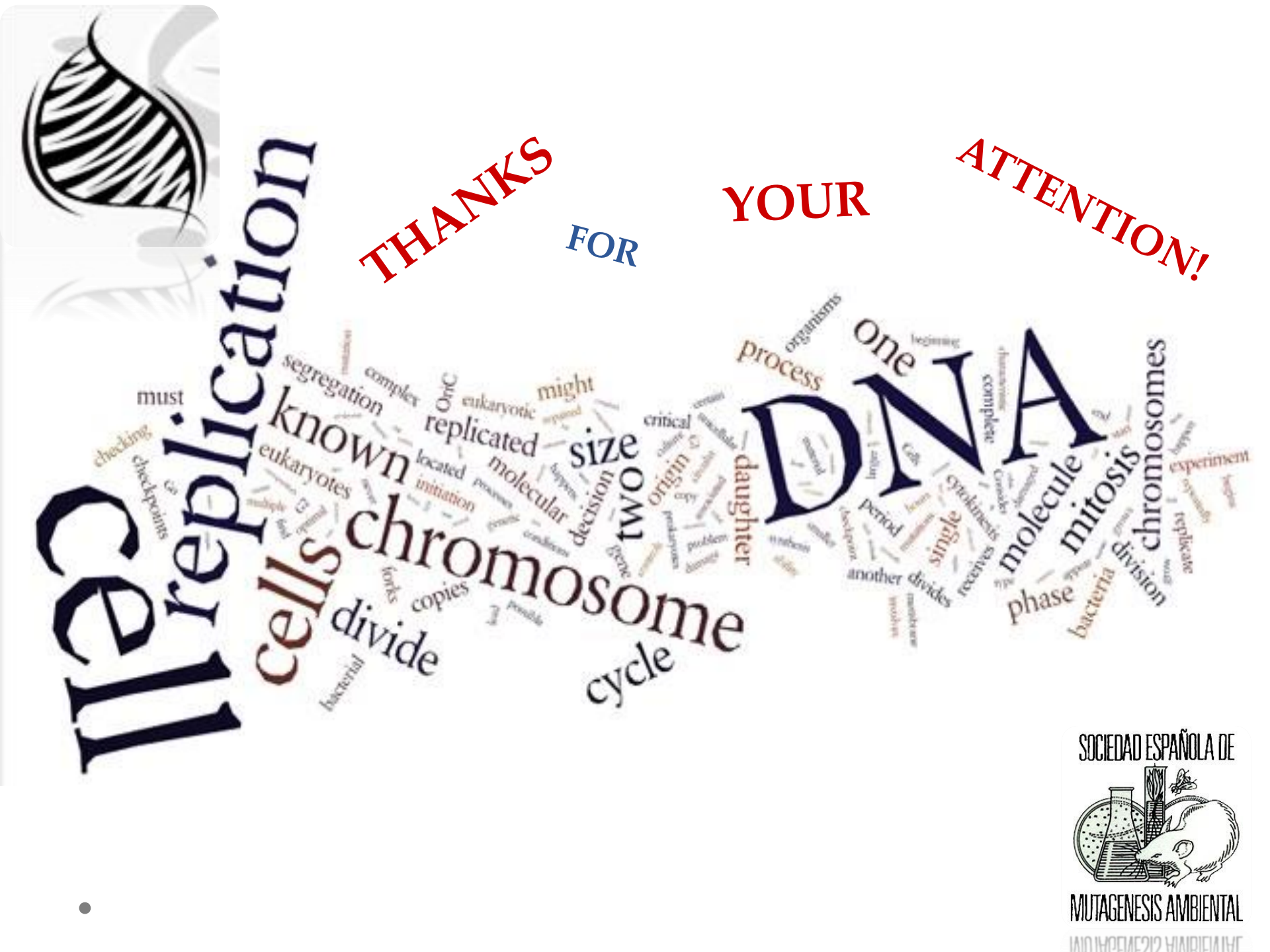

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