

# HIV antiretrovirals gene expression biomarkers of genotoxicity

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# Nucleoside reverse transcriptase inhibitors (NRTs)

- Currently used for the therapy of AIDS
- Most commonly combined in one pill
- AZT, ddI, d4T, 3TC mostly used
- Mimic DNA nucleosides (ATCG)
- Typically chain terminators
- Inhibit HIV-1 reverse transcriptase

# AZT preclinical genotoxic profile

Bacterial gene mutation	-
Mamalian gene mutation	+
L5178 YTK +/- assay	+
Clastogenicity assay	+
Micronucleus assay	+
SCE assay	+
Cell transformation	+
Carcinogenicity	+



# Genotoxicity after exposure to AZT *in utero*

## ■ Newborn monkeys

*Olivero Mutat Res. 2008.Review*



Incorporation into DNA at birth

Shortened telomeres

Micronucleus

Supernumerary centrosomes

Aneuploidy

## ■ Newborn infants

Incorporation into DNA

GPA mutagenesis

Micronucleus

Heterochromatin dispersion

*Olivero AIDS 1999*

*Escobar Environ Mol Mutag 2007*

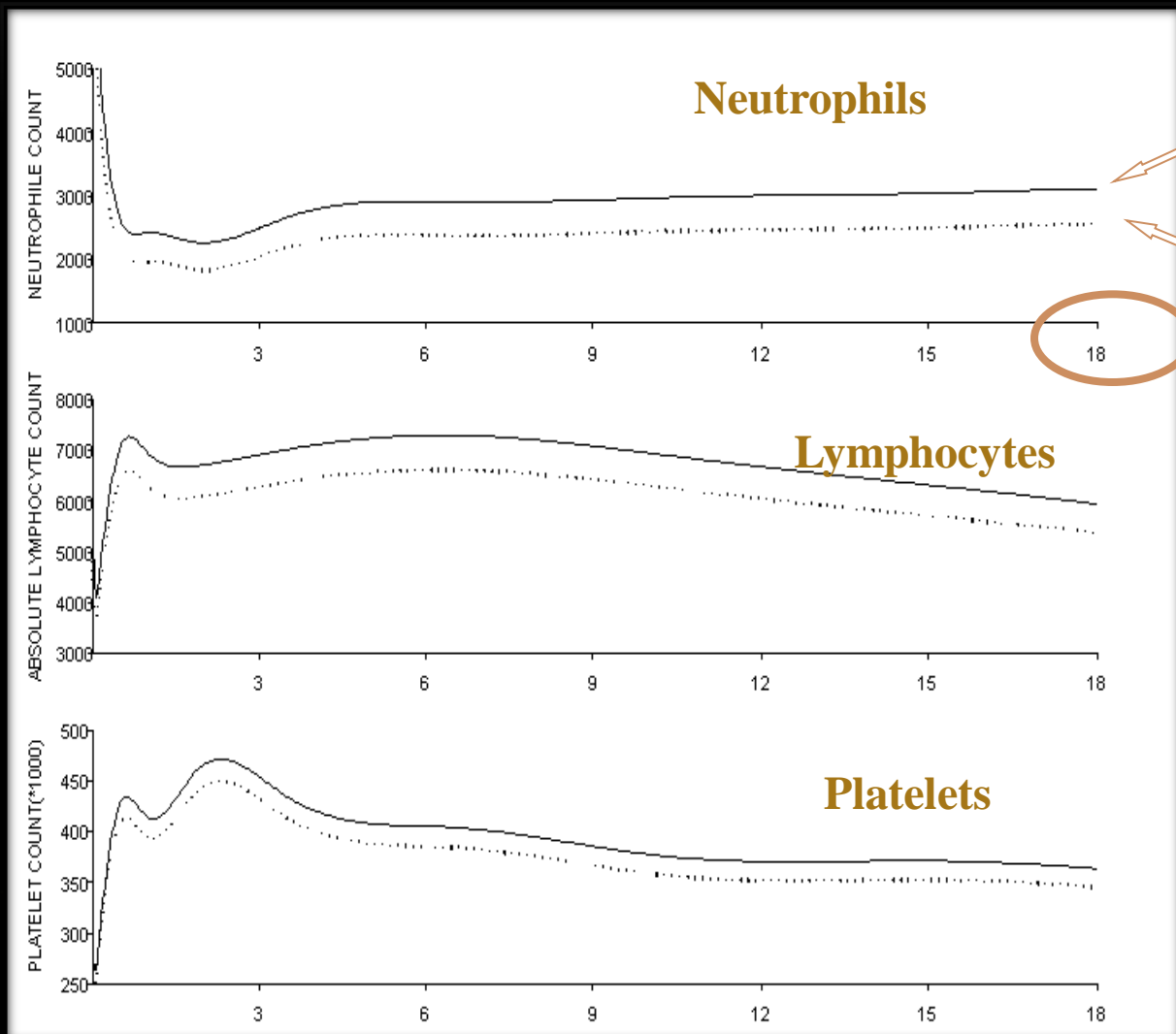
*Witt Environ Mol Mutagen 2007*

*Sho Antiviral Th 2007*



# Defective hematopoiesis is observed in a cohort of French uninfected children exposed to AZT during gestation

*Le Chenadec AIDS 2003*



AZT -

AZT +

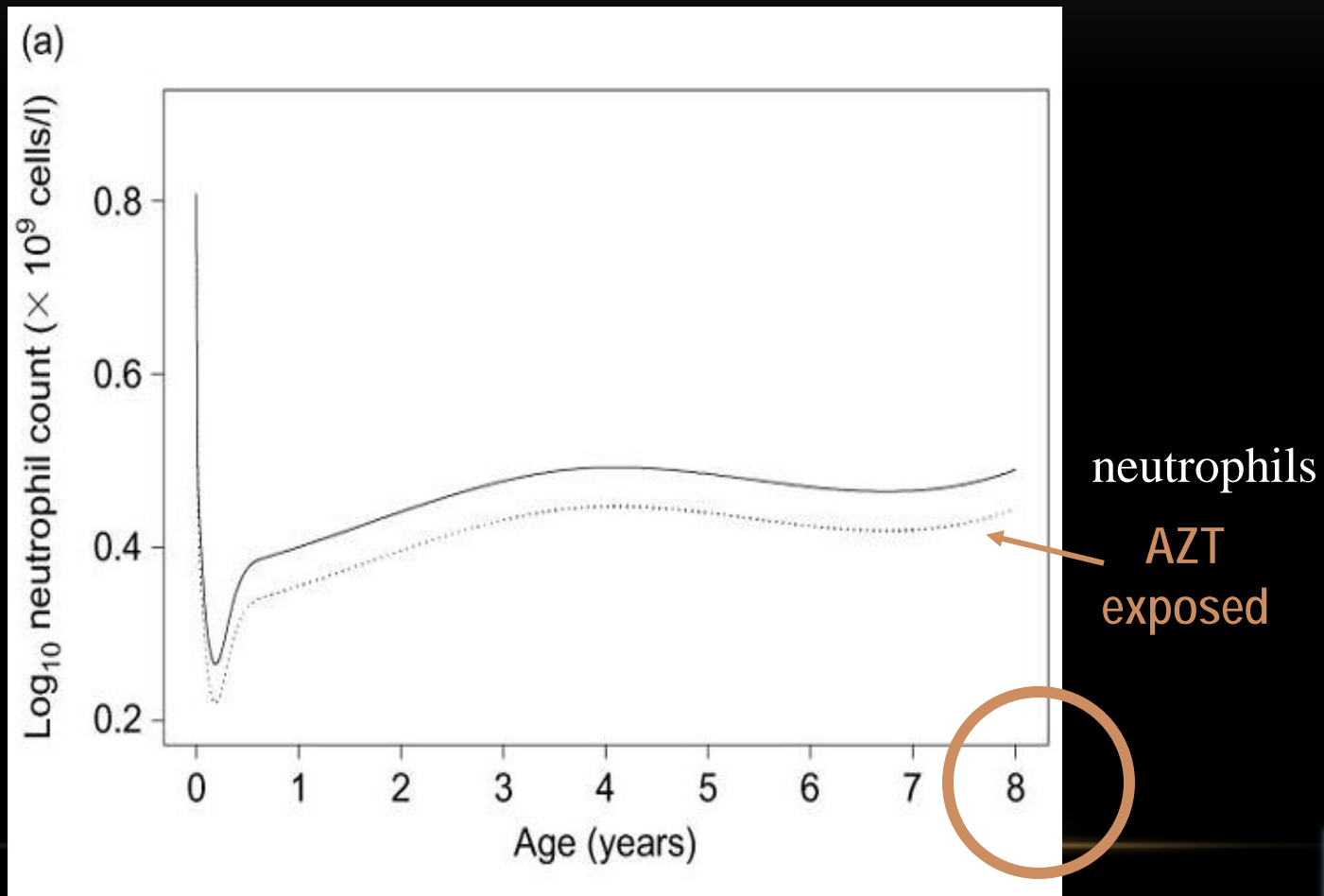
French Cohort  
1986-2002

4249  
uninfected children

>21 000  
*blood counts*



# Neutrophils count in perinatally AZT exposed children until 8 years



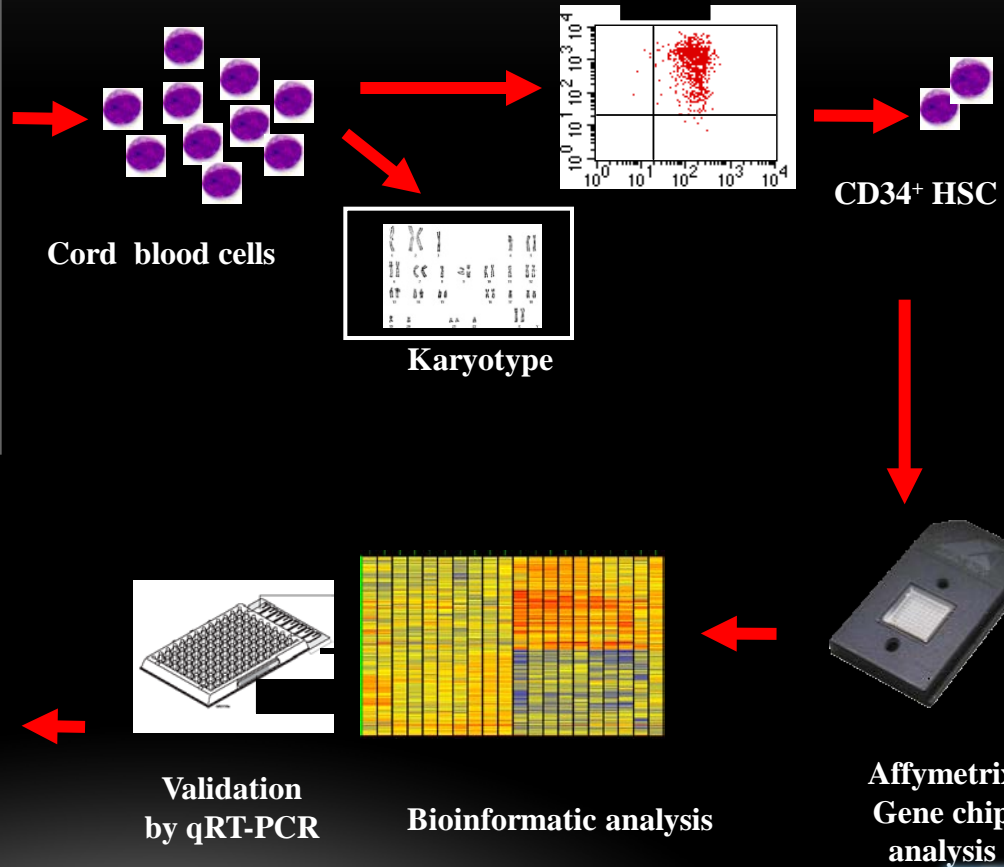
*Is there hematopoietic stem  
cell compromise?*



# Gene expression in CD34<sup>+</sup> cord blood cells of AZT exposed newborn



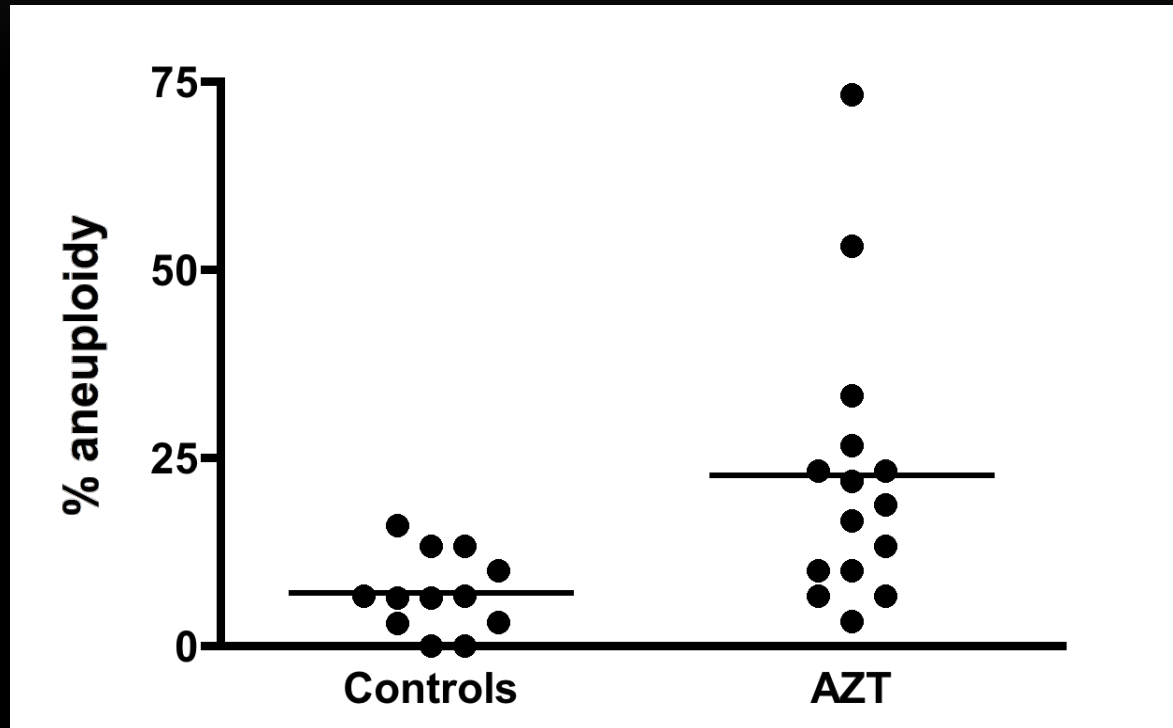
Uninfected AZT-exposed  
and control newborn



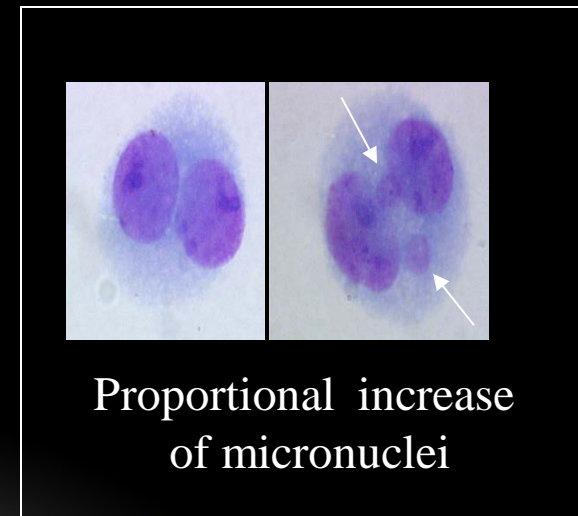
Transcriptional  
profiling analysis  
of CD34<sup>+</sup>  
hematopoietic  
stem cells



# Increased aneuploidy in cord blood lymphocytes from AZT-exposed newborn

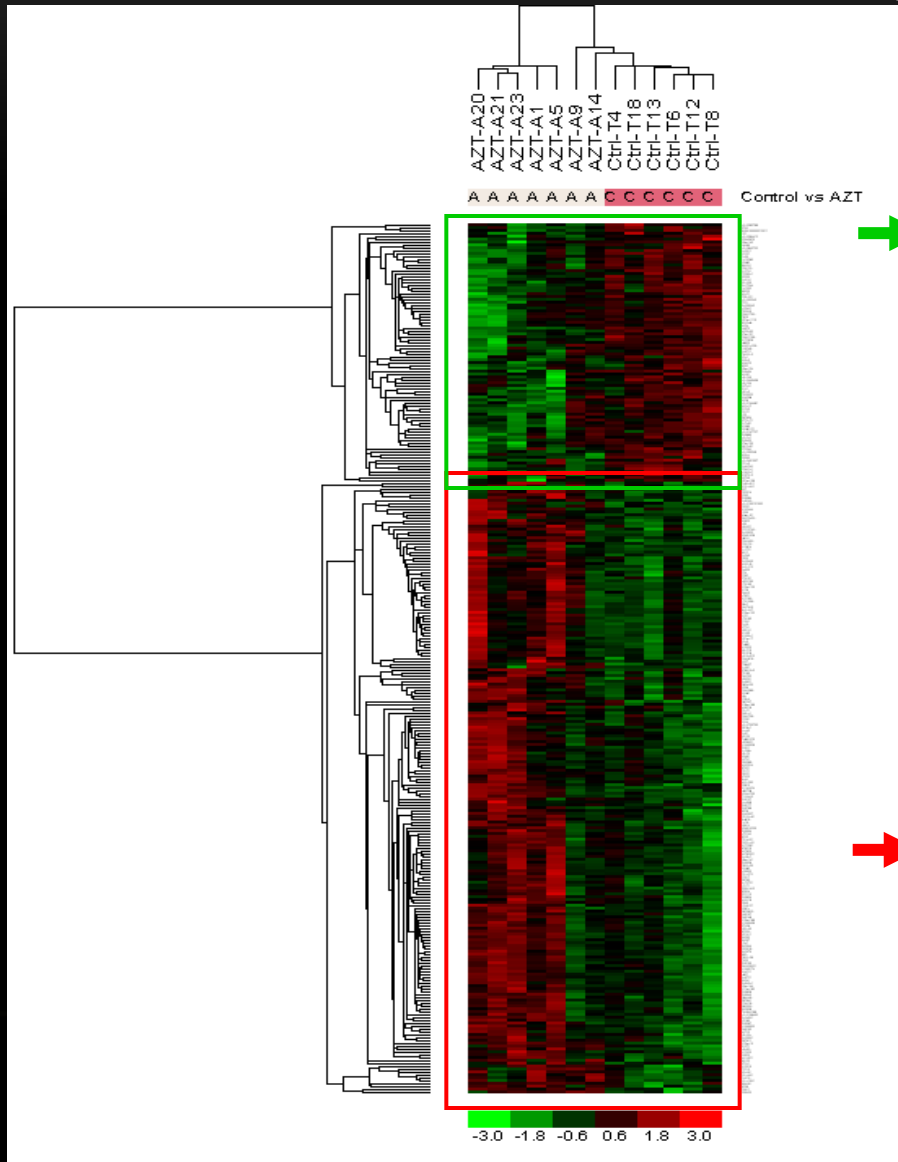


Median (%): **6.6** vs **18.7**  $p < 0.001$



# Gene expression profiles control vs AZT-exposed CD34+

*Andre-Schmutz J Infect Dis 2013;208:235.*



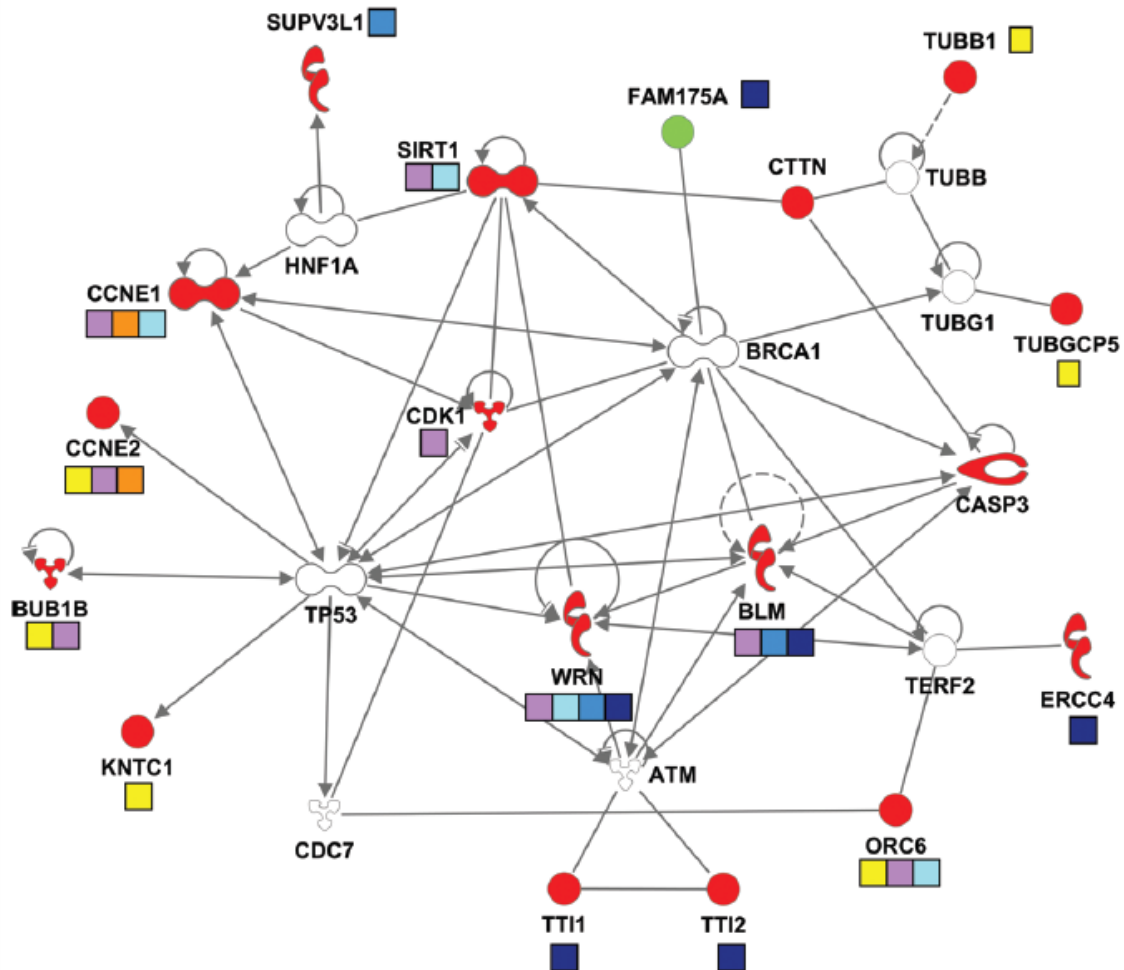
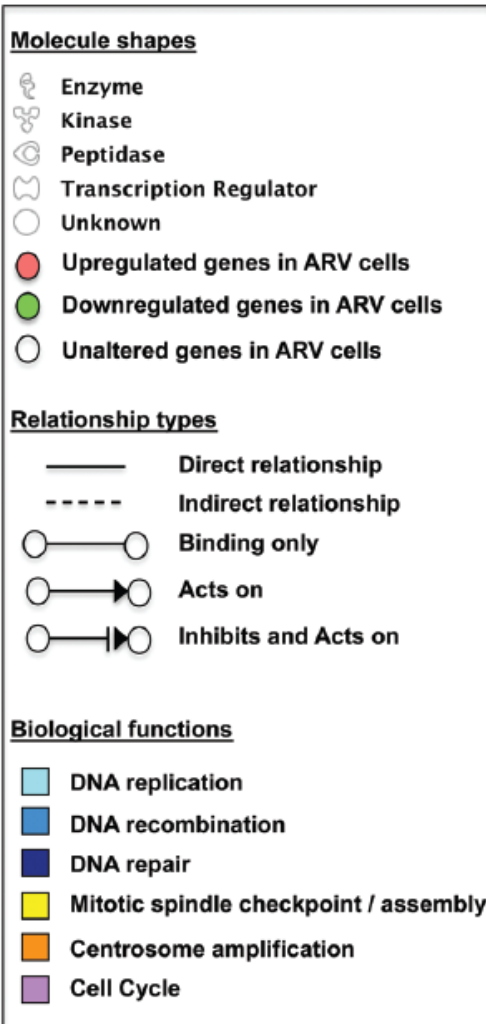
**90 genes down-regulated in ARV-treated cells**

**213 genes up-regulated in ARV-treated cells**

*P value 0.05 Fold Change 1.5*



# Ingenuity pathways



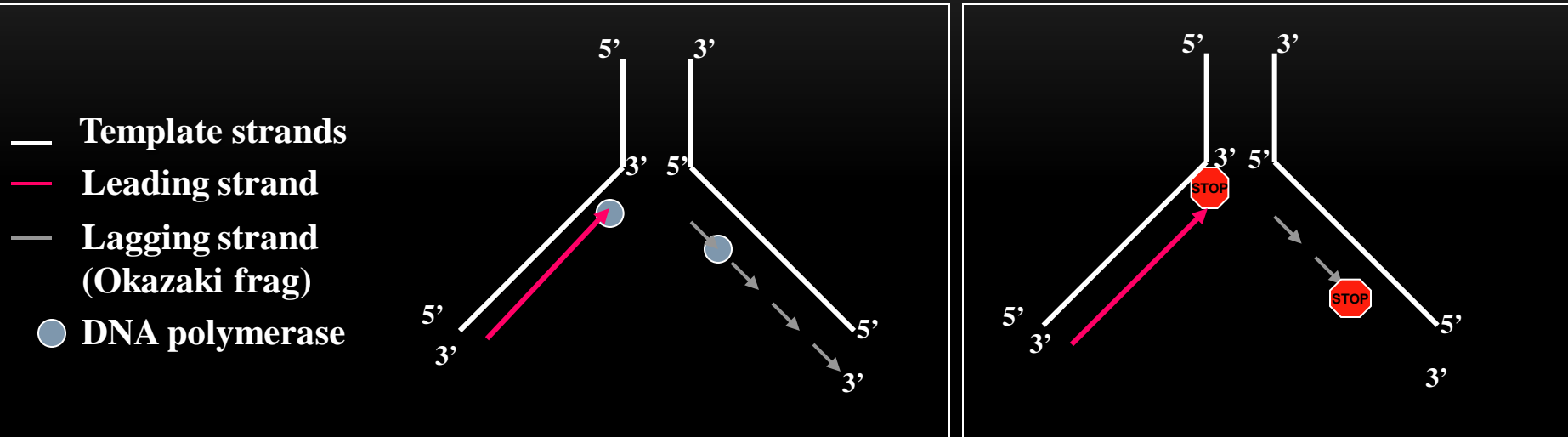
# Genotoxicity of antiretroviral therapy

## AZT

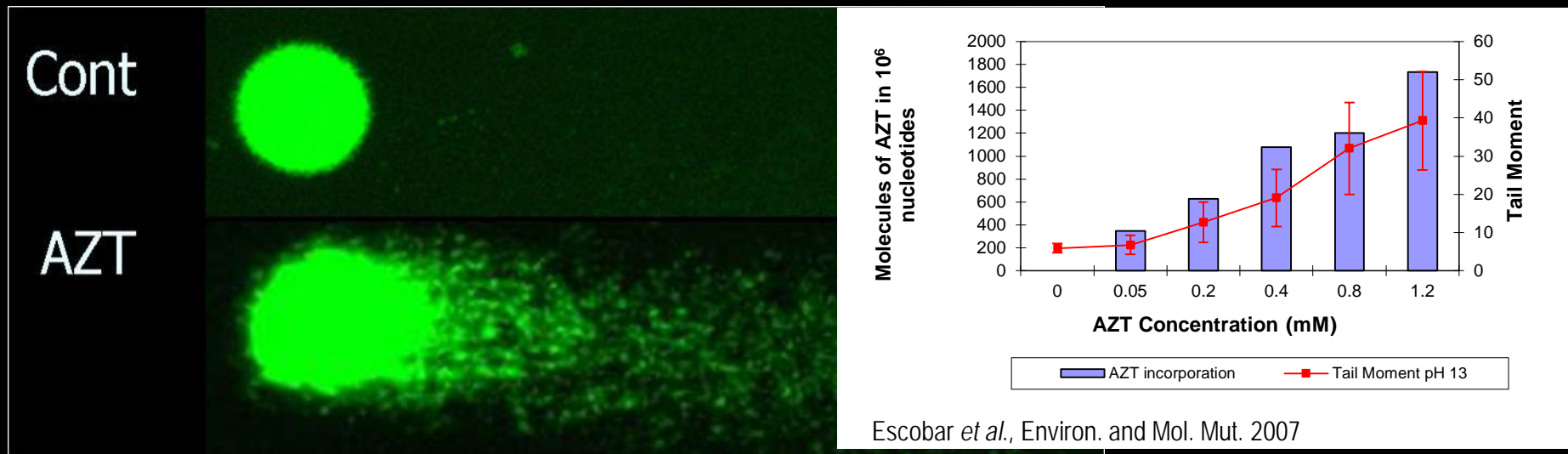
- ✓ DNA chain terminator
- ✓ Works as aneugen
- ✓ Induces centrosomal amplification
- ✓ Inhibits tubulin polymerization



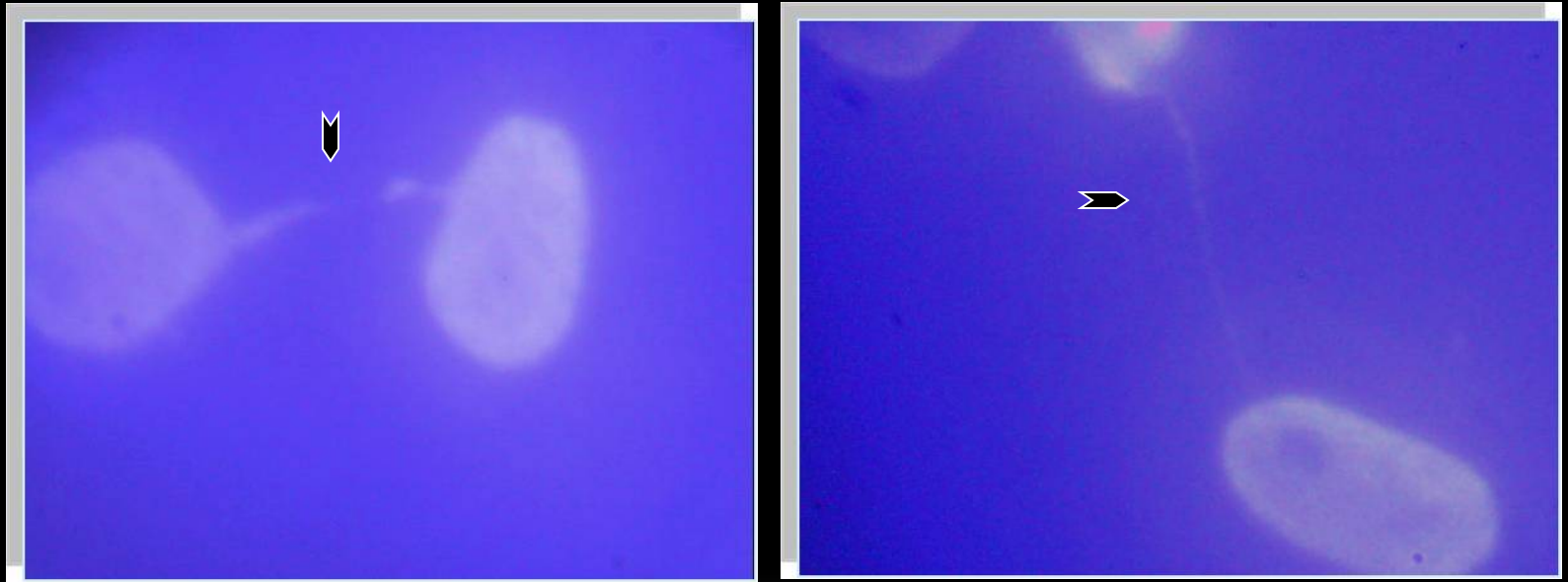
# AZT generates DNA fragments



## AZT incorporates into DNA and generates DNA fragments

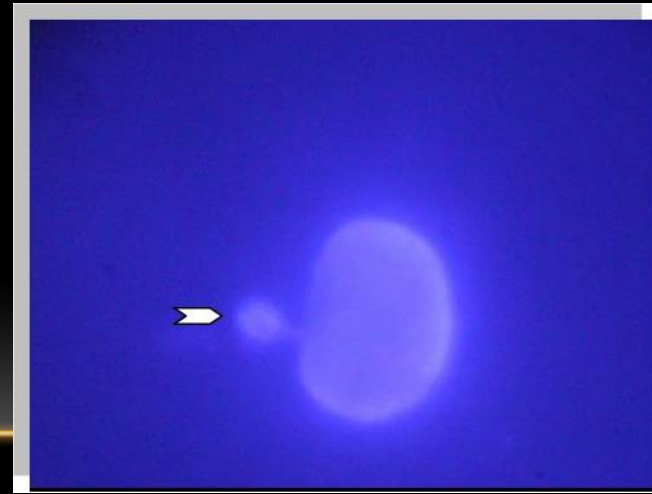
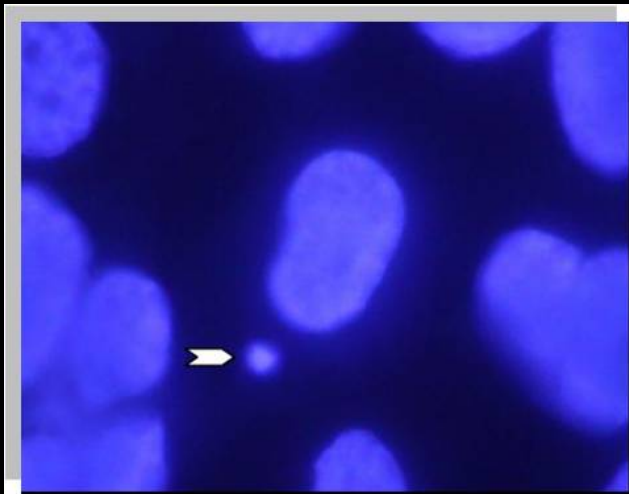
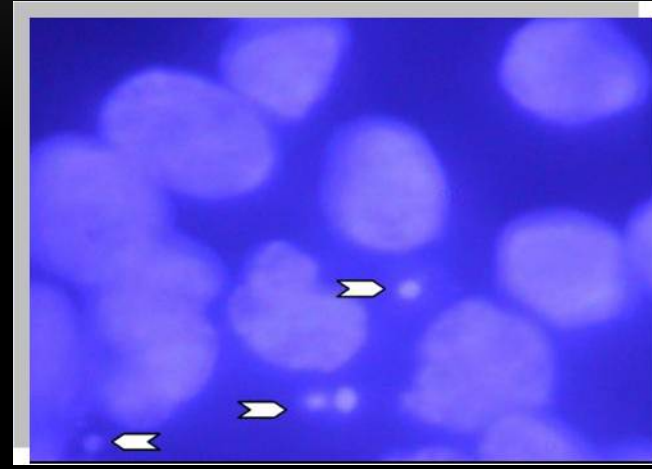
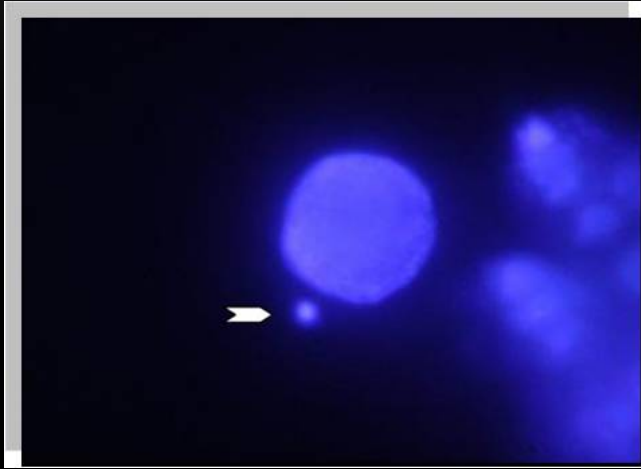


Telomeric disruption (by AZT-DNA incorporation)  
produces end to end fusions identified as chromatin bridges



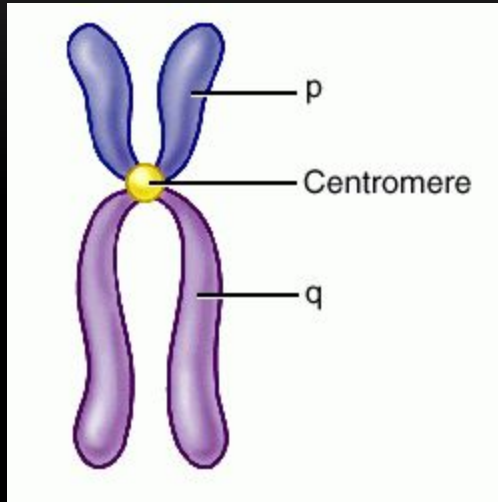
*HeLa cells, 800  $\mu$ M AZT 24 hours*

# Chromatin bridges and chromosome breaks induce micronuclei in cells in culture

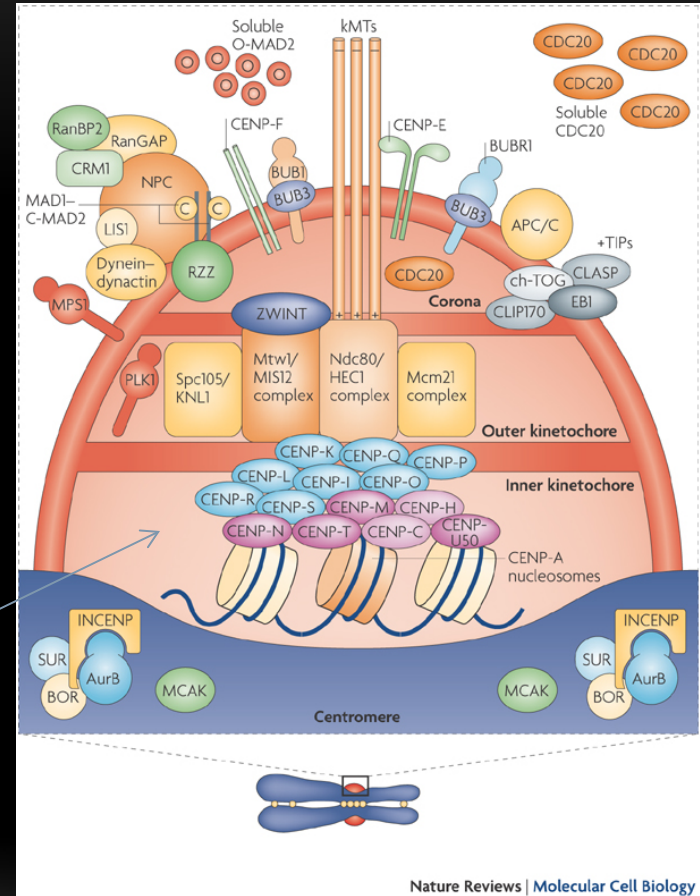
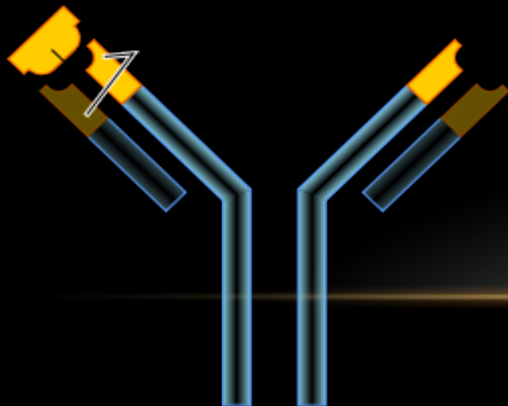


*HeLa cells, 800  $\mu$ M AZT 24 hours*

# The centromere, a complex structure



CREST  
antibodies



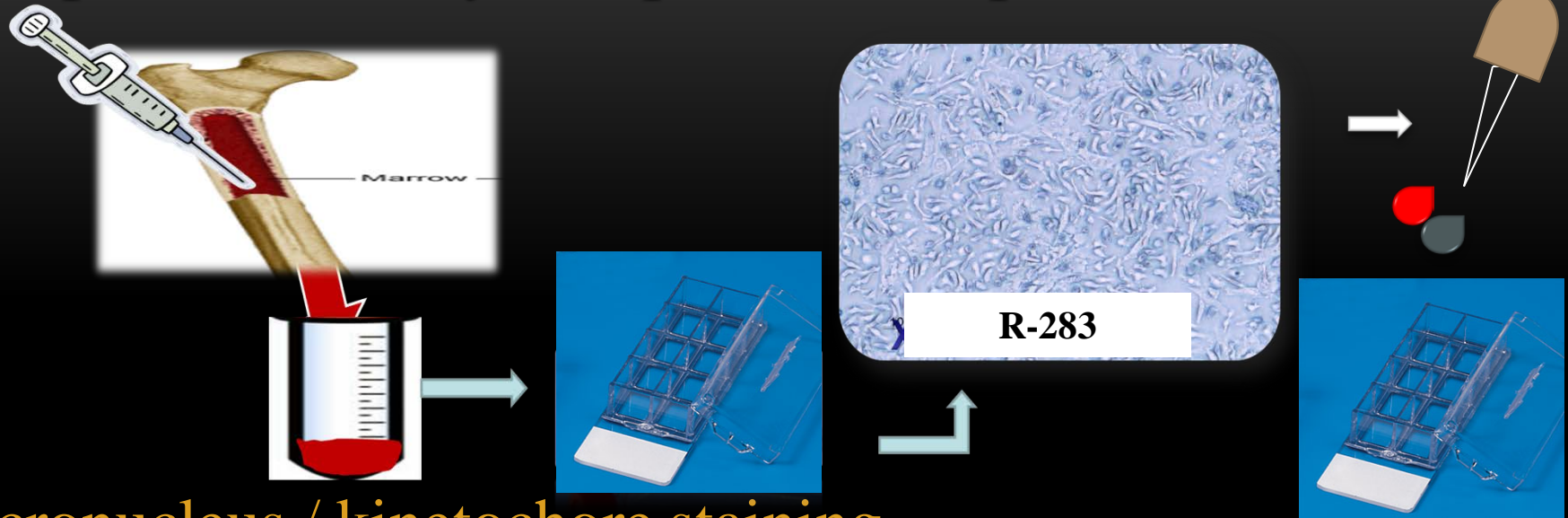
# Aneuploidy induced by AZT in vitro



(Borojerdi et al, Mutat. Res .2009)

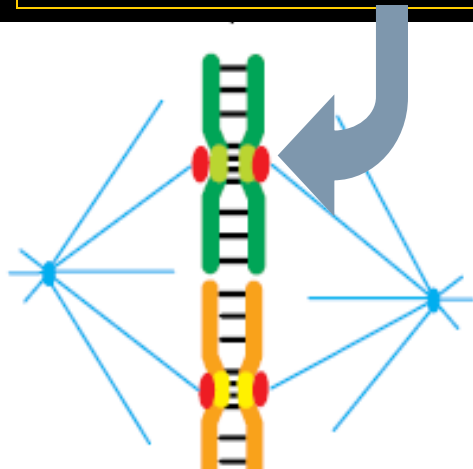


# *E. patas* monkeys, experimental procedures

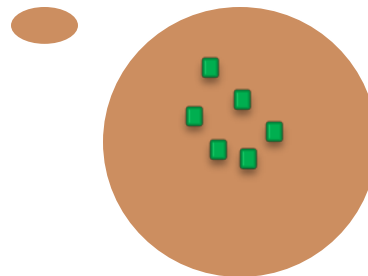


## Micronucleus / kinetochore staining

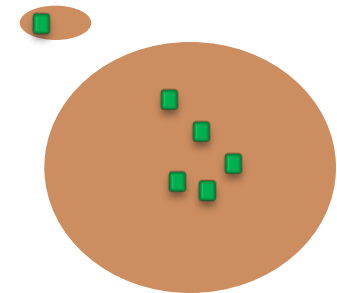
CREST antibody



● kinetochore

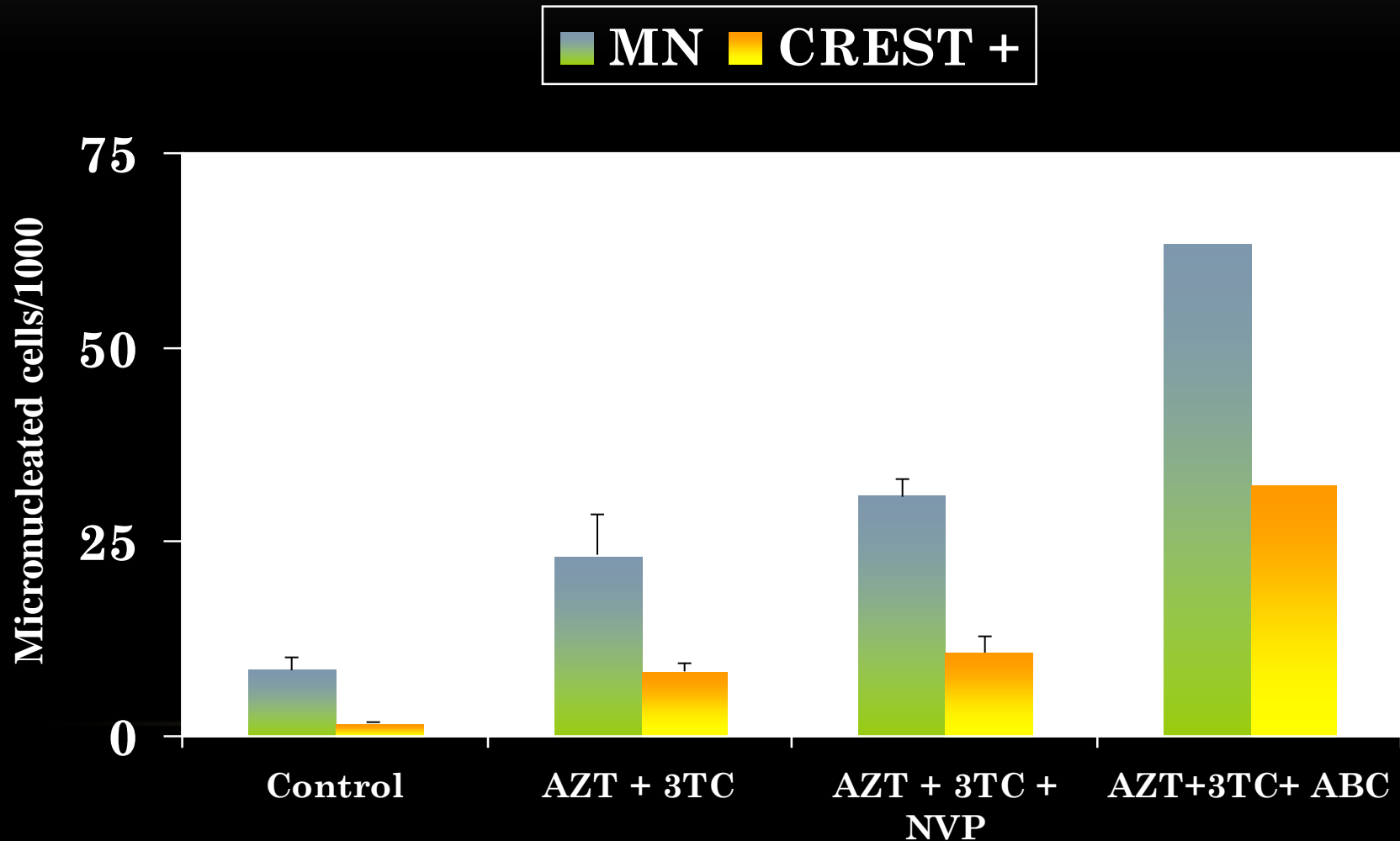


CREST -



CREST +

# Micronuclei formation and CREST staining status in monkeys exposed in utero to NRTIs



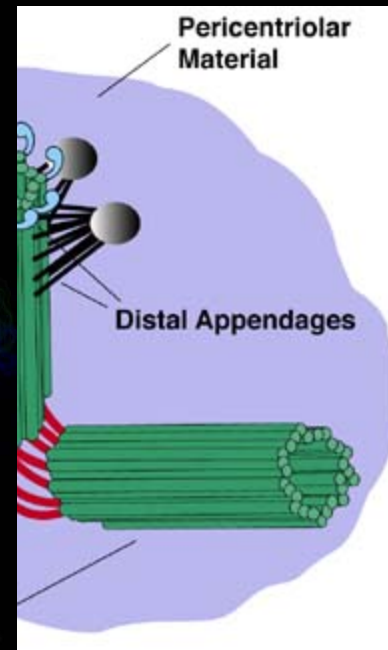
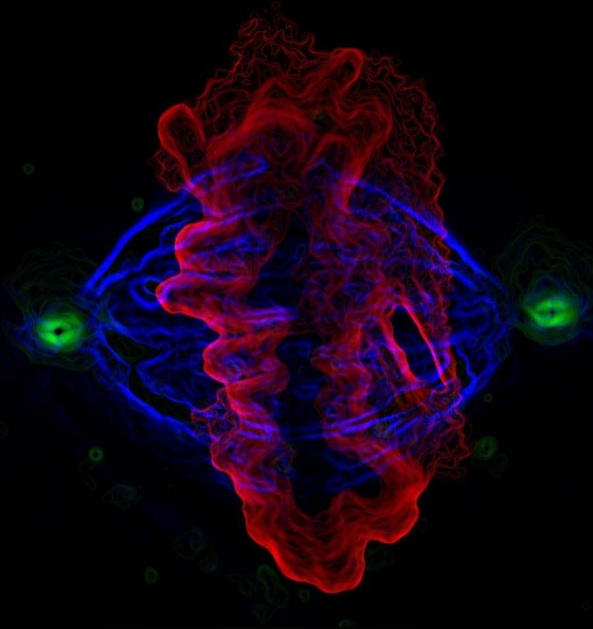
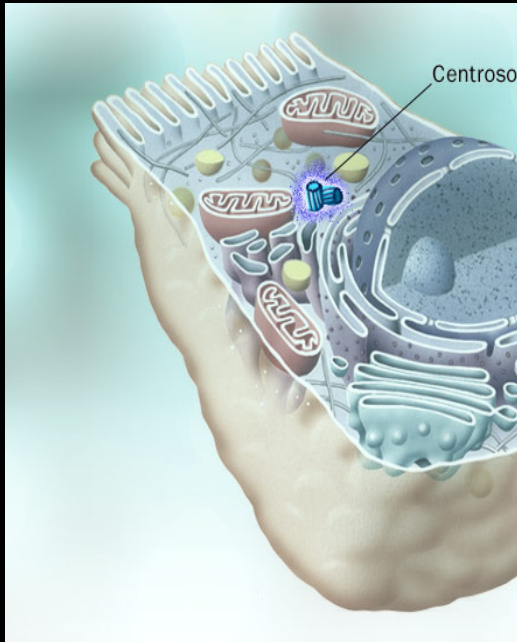
# Aneuploidy induced by AZT in vitro



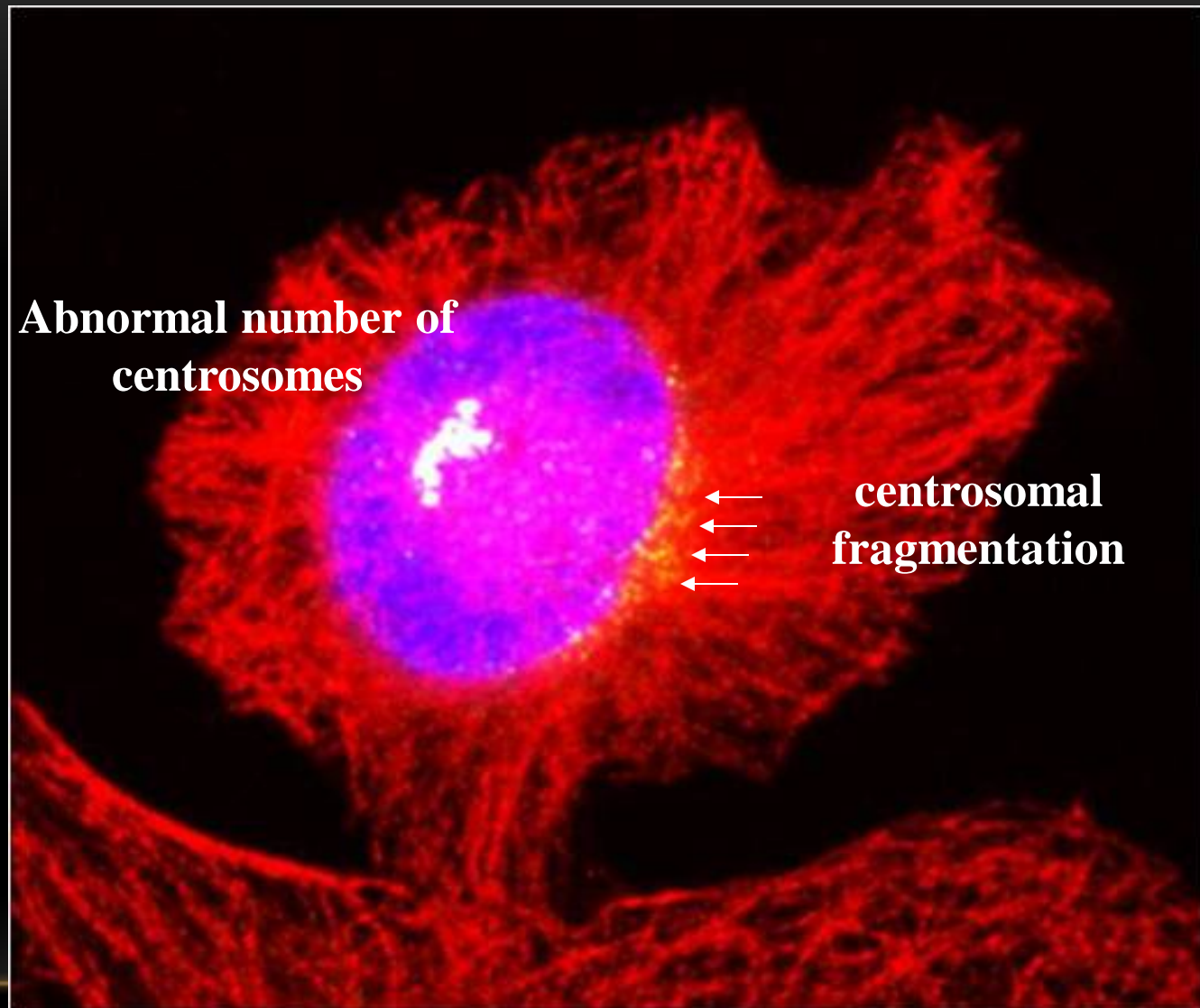
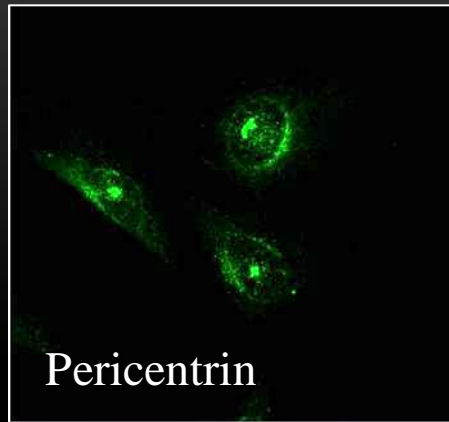
(Borojerdi et al, Mutat. Res .2009)



# The centrosome commands cell division

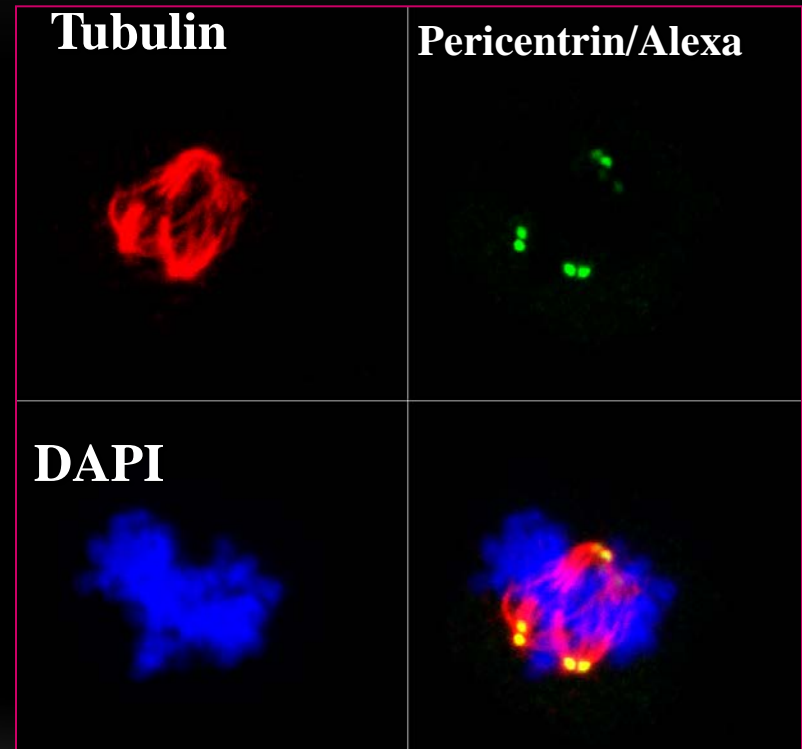
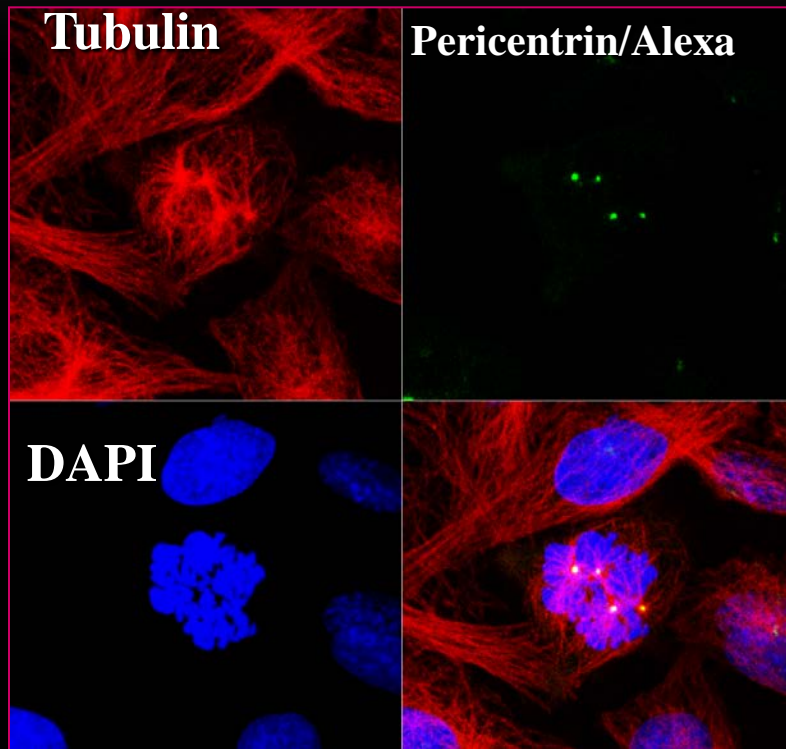


# Localization of centrosomes and characterization of integrity in CHO cells



800  $\mu$ M AZT

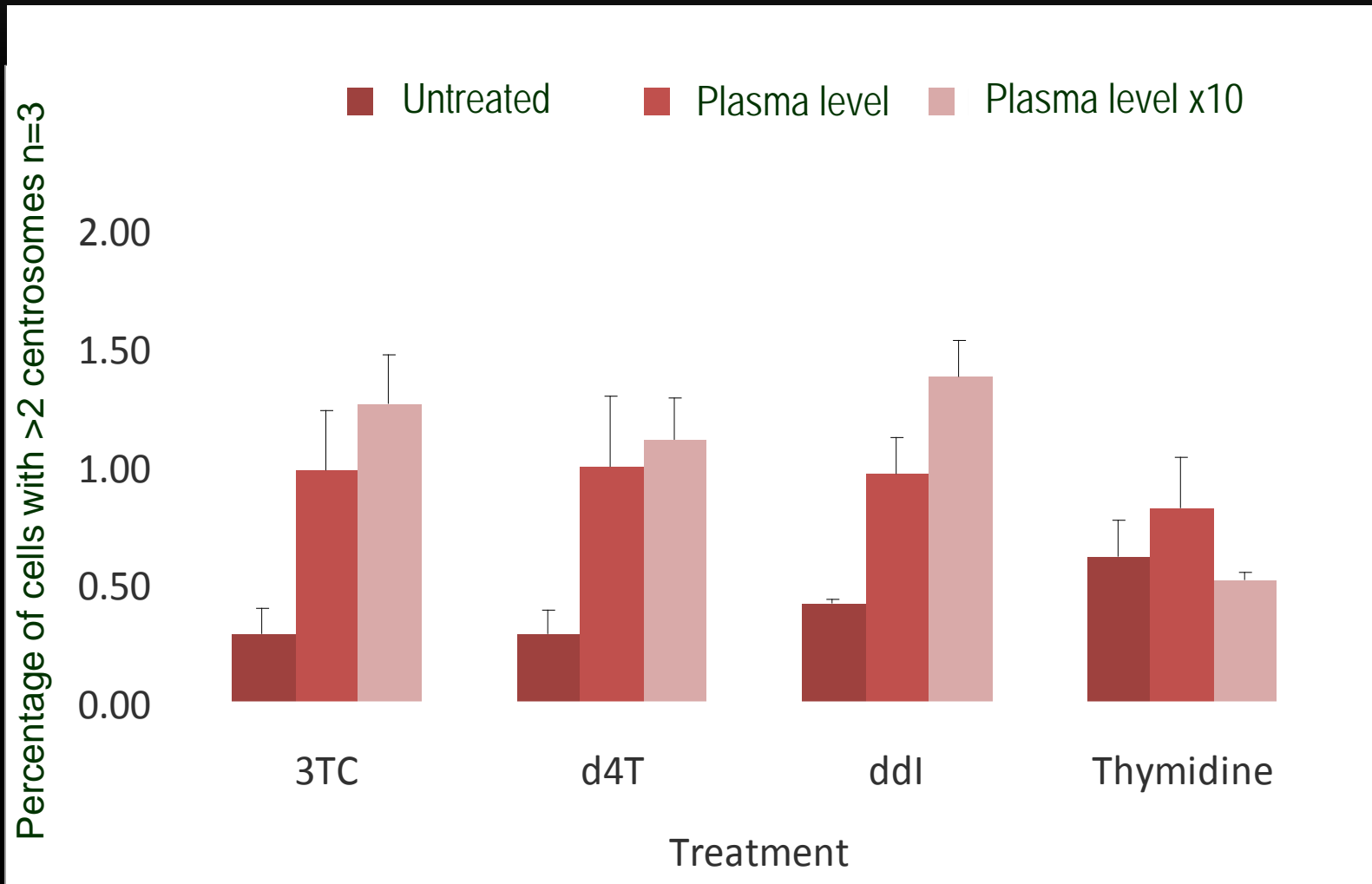
# Abnormal mitotic spindles, extra centrosomes (CHO cells, exposed to NRTIs)



(Yu et al., EMM 2009)



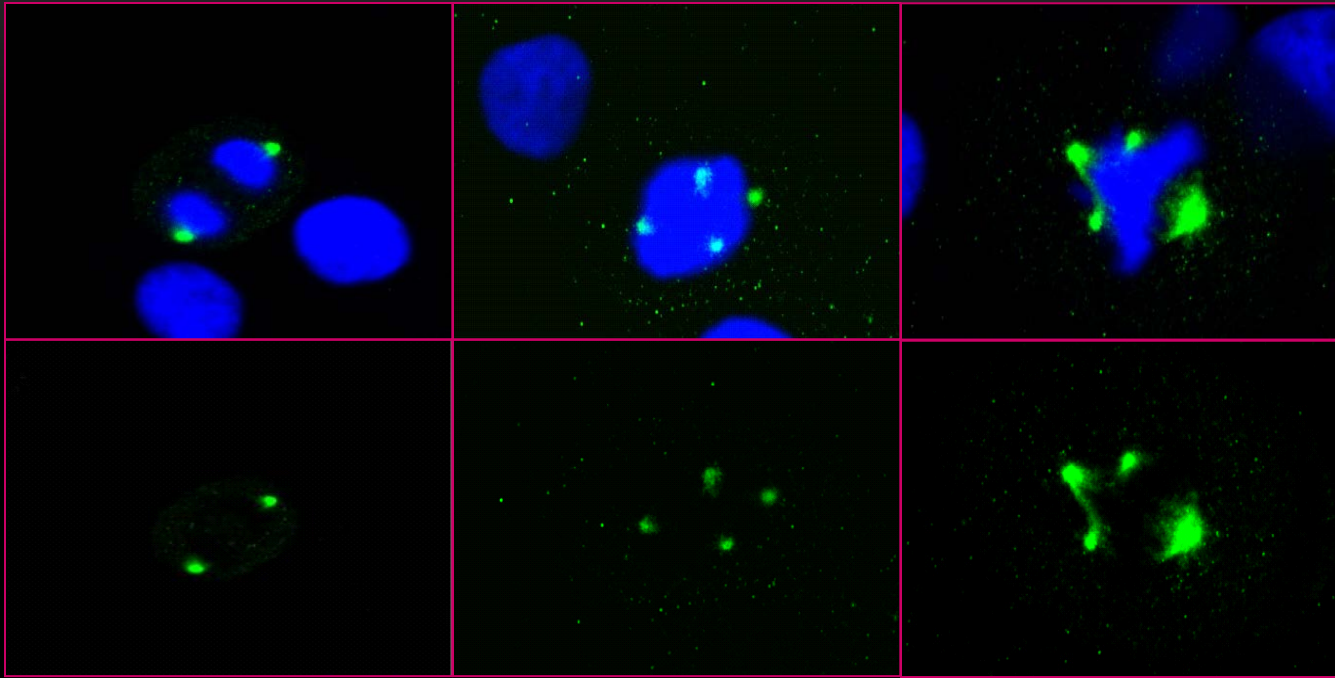
# NRTIs, but not thymidine, induced an increase in the percent of abnormal CHO cells (containing extra centrosomes)



(Yu et al., EMM 2009)

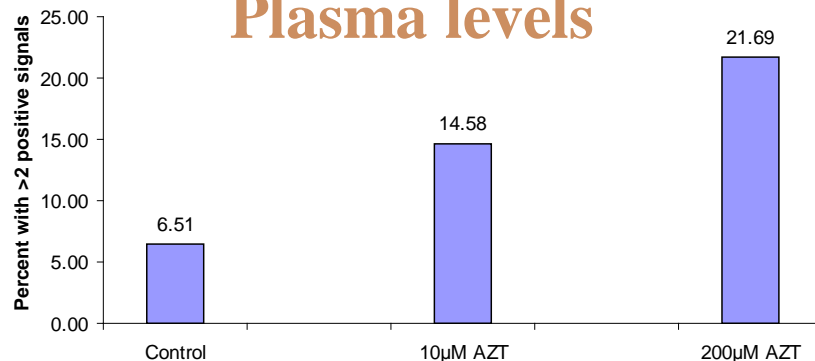


# Abnormal mitotic spindles



Aurora A, a protein kinase, is localized in the centrosome and spindle poles

## Plasma levels

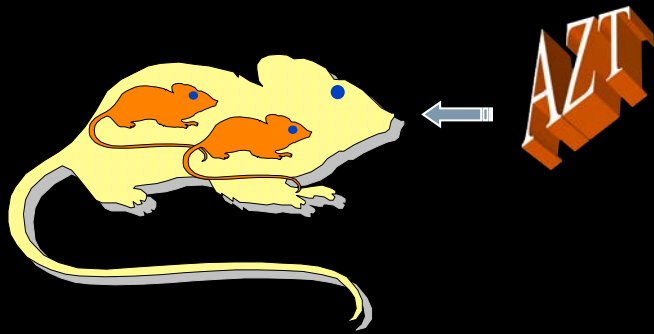


An increase in Aurora positive signals correlated with AZT dose

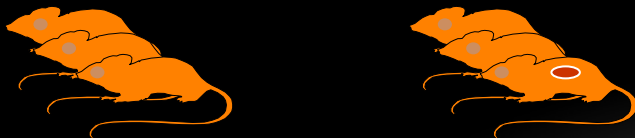
(Borojerdi et al, Mutat. Res .2009)



# AZT is a transplacental carcinogen in rodents

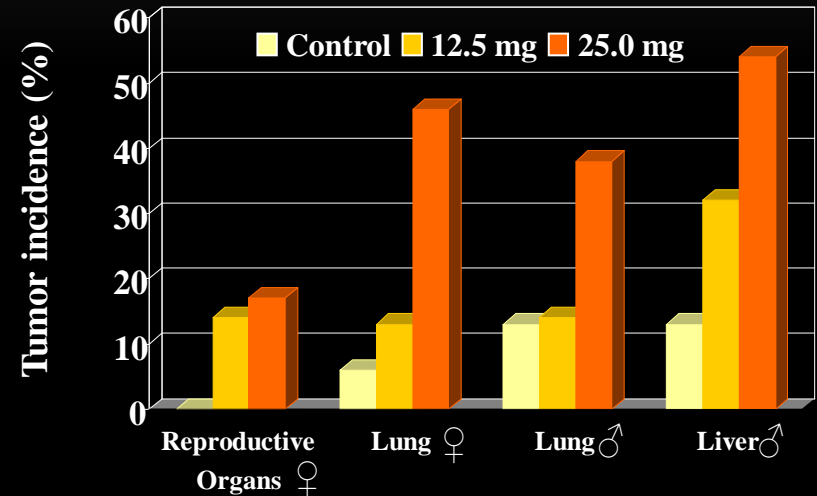


Offspring grown  
to adulthood without  
further exposure



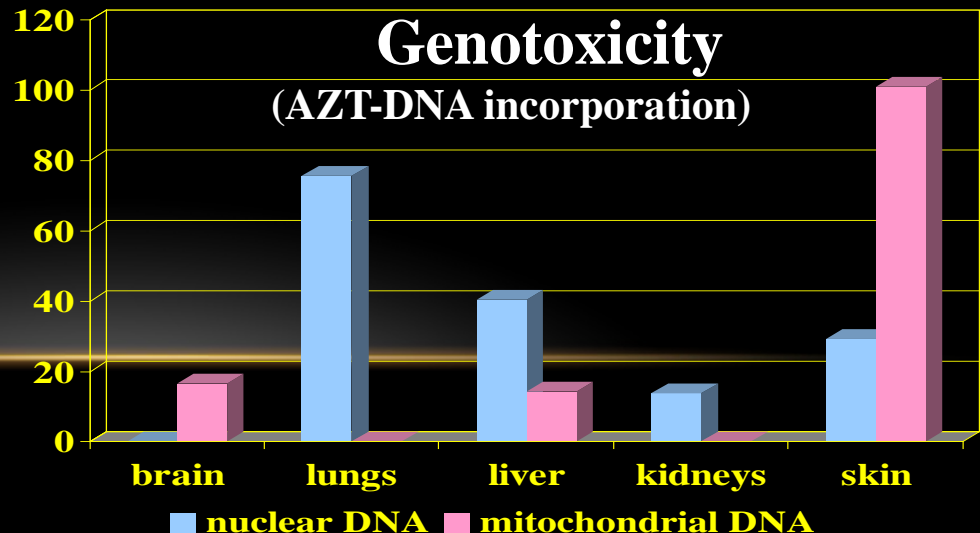
One Year Study  
*Olivero et al, JNCI 1997*

## Tumorigenicity

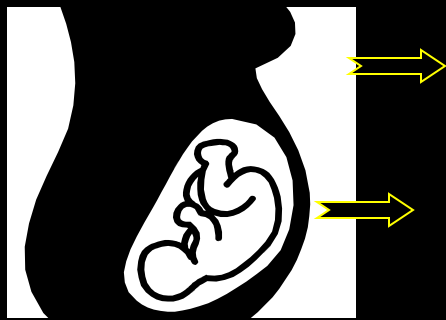


Molecules of AZT/10<sup>6</sup> nucleotides

## Genotoxicity (AZT-DNA incorporation)



# Incorporation of AZT in human DNA of infants exposed in utero



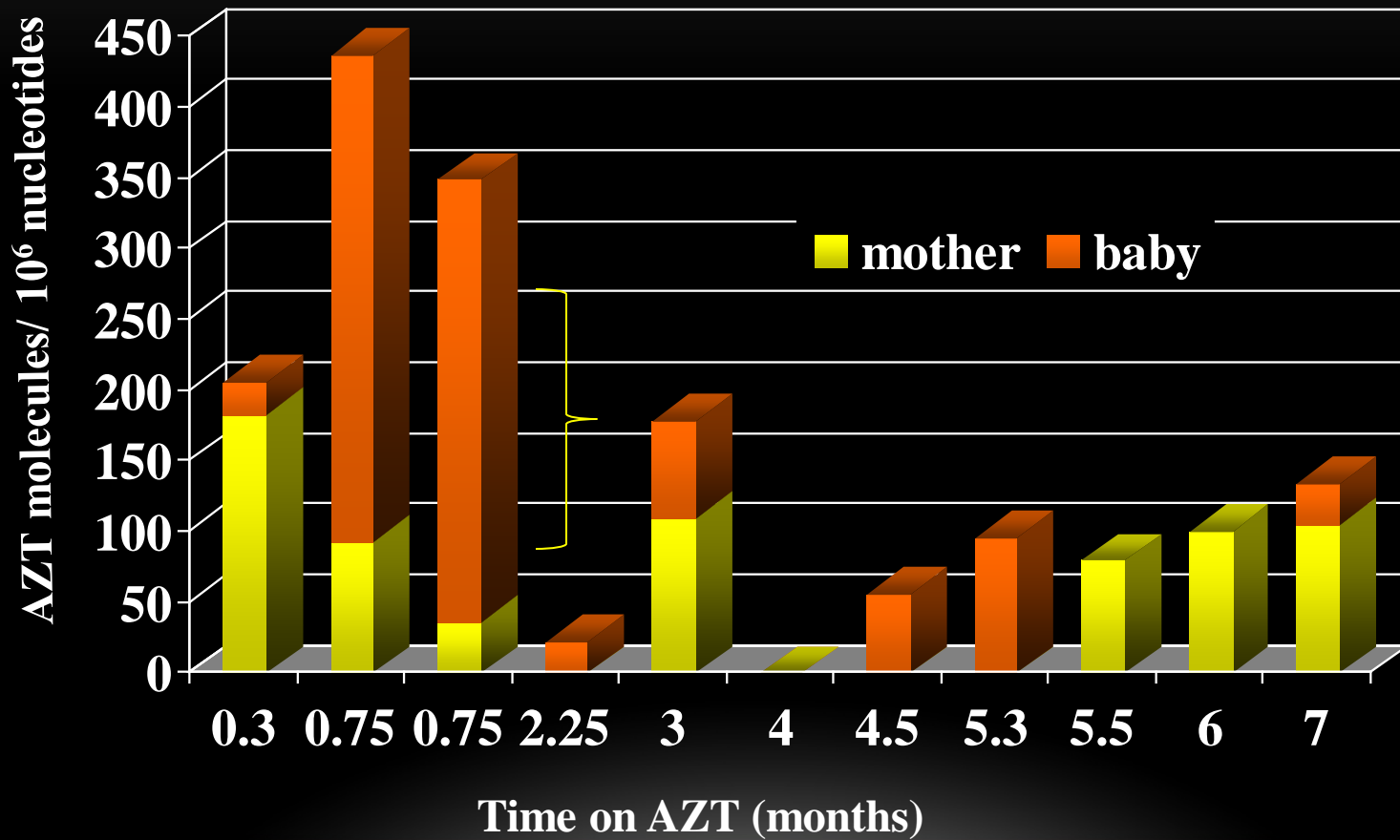
Maternal peripheral blood

Fetal umbilical cord blood  
(Risk-exposure markers)

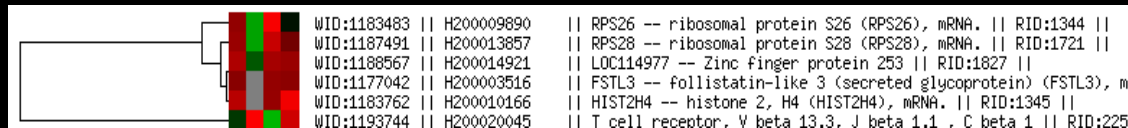
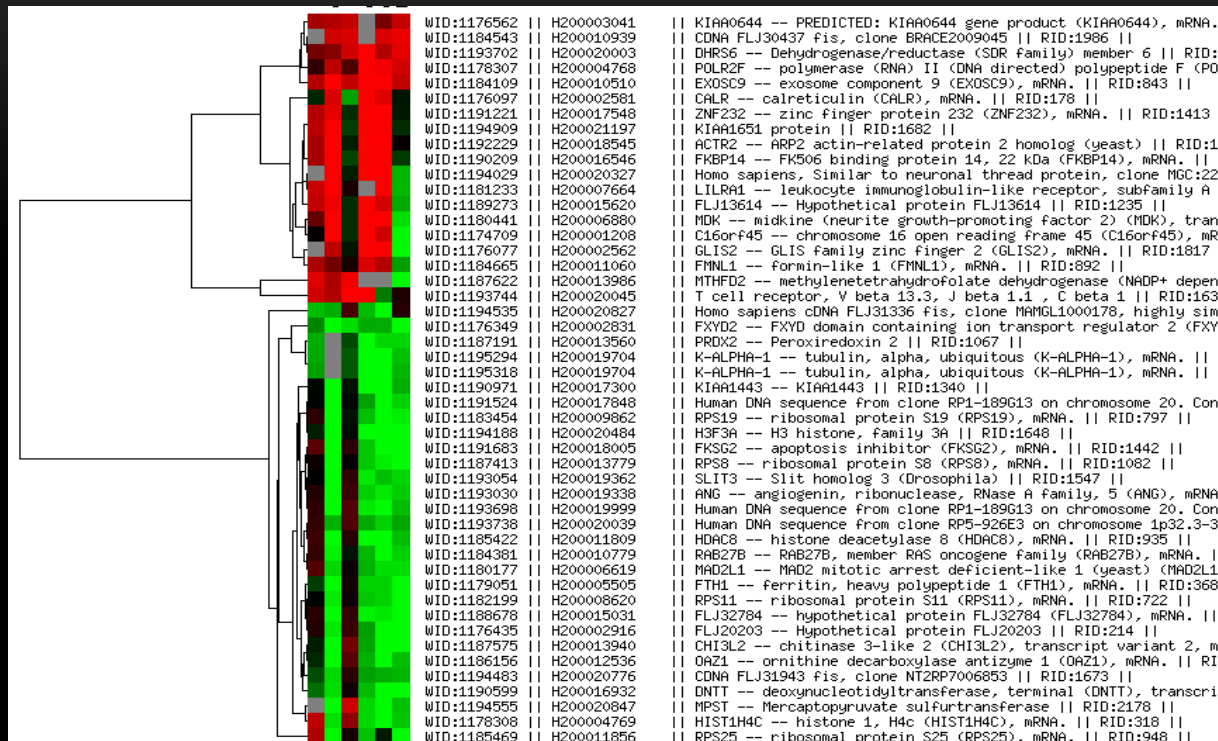
- **DNA extraction from PBMCs**
- **AZT-DNA incorporation by RIA**



# AZT incorporates into DNA of human blood cells



# AZT-induced gene expression *in vitro* in lymphoblastoid cells



# Molecular signature

- Looking for a group of genes or microRNAs or metabolic pathways that are characteristics of the disease studied

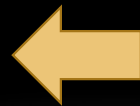
To elucidate expression of specific elements involved in kinetochore attachment and activation and in centrosomal structure and function. Additionally, information obtained from microRNA arrays is requested as a valuable tool to identify alternative mechanisms related to tumorigenesis.

- AZT treated cells were studied to explore the changes in gene expression, miRs and methylation

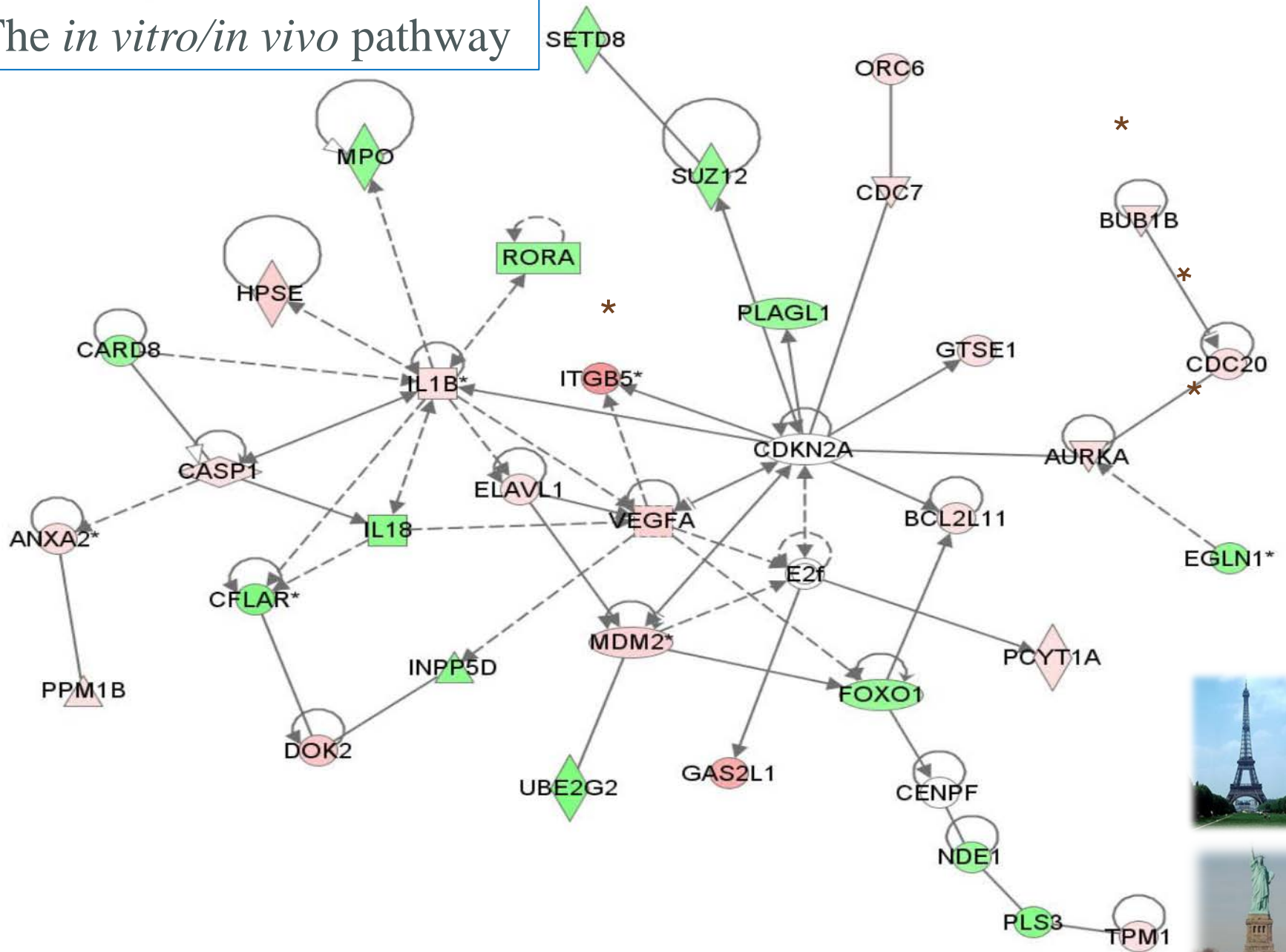


MCF 10 A human mammary normal epithelial cells

0, 10 and 100 M AZT  
24, 48 and 72 hr



## The *in vitro/in vivo* pathway

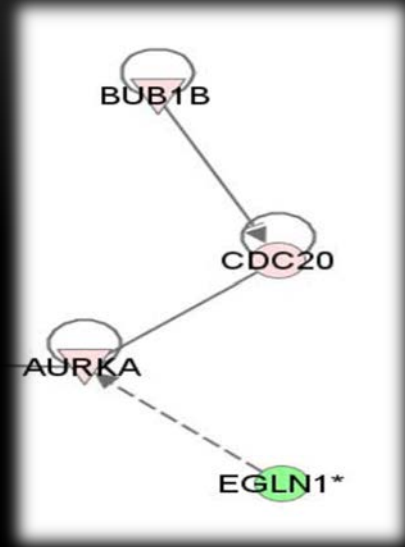


# Molecular signature

- A group of genes or microRNAs or metabolic pathways that are characteristics of the disease studied
- A group of genes expression pathways that are specific responses to NRTI exposure and are common to *in vitro* and *in vivo* systems

# Molecular signature

- Spindle-assembly checkpoint signaling for correct chromosome alignment.
- Nuclear movement prior to anaphase and chromosome separation.
- Centrosome maturation by moderating the recruitment of proteins, which are themselves essential for accumulating microtubule spindle components, such as gamma-tubulin. Separation of centrosomes.



# Gene, mRNA and protein expression in AZT treated cells

PROTEIN*	miRNA**	DNA**
HSPD1 (HSP-60)	<i>hsa-miR-139-5p</i>	SCG5
NME1 (NDPK-A)	<i>hsa-miR-770-5p</i>	SERPINB7
SOD1 (SOD)		KRT6B
GRP58 (PDI-A3)		PGLYRP2
STMN1 (Stathmin)		OSBPL10
		TP53I3
		CCDC49
		VIPR1

\*D'Andrea et al, Proteome Science, 2006

\*\*Dan Edelman, CMPC, CCR



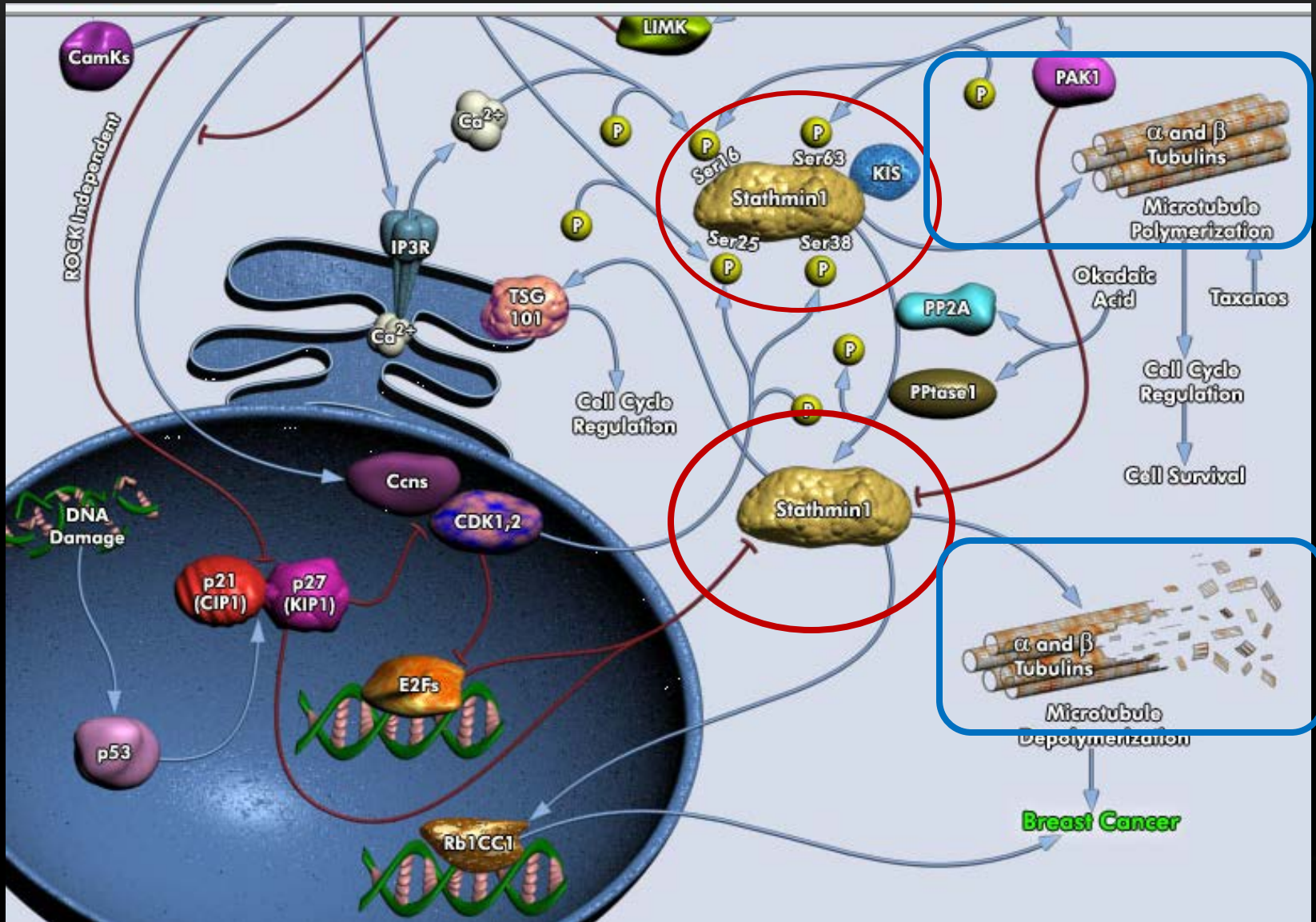
# Stathmin -1 is a target of hsa-miR-770-5p

There are 212 predicted targets for hsa-miR-770-5p in [miRDB](#)

Target Detail	Target Rank	Target Score	miRNA Name	Gene Symbol	Gene Description
<a href="#">Details</a>	1	100	hsa-miR-770-5p	<a href="#">ZDHHC11B</a>	zinc finger, DHHC-type containing 11B
<a href="#">Details</a>	2	99	hsa-miR-770-5p	<a href="#">ZNF765</a>	zinc finger protein 765
<a href="#">Details</a>	3	99	hsa-miR-770-5p	<a href="#">ZDHHC11</a>	zinc finger, DHHC-type containing 11
<a href="#">Details</a>	4	95	hsa-miR-770-5p	<b>STMN1</b>	stathmin 1
<a href="#">Details</a>	5	93	hsa-miR-770-5p	<a href="#">ZNF117</a>	zinc finger protein 117
<a href="#">Details</a>	6	90	hsa-miR-770-5p	<a href="#">ZNF138</a>	zinc finger protein 138
<a href="#">Details</a>	7	90	hsa-miR-770-5p	<a href="#">ZNF107</a>	zinc finger protein 107
<a href="#">Details</a>	8	89	hsa-miR-770-5p	<a href="#">BTG1</a>	B-cell translocation gene 1, anti-proliferative
<a href="#">Details</a>	9	89	hsa-miR-770-5p	<a href="#">ZNF99</a>	zinc finger protein 99
<a href="#">Details</a>	10	86	hsa-miR-770-5p	<a href="#">MYO6</a>	myosin VI
<a href="#">Details</a>	11	85	hsa-miR-770-5p	<a href="#">NTRK2</a>	neurotrophic tyrosine kinase, receptor, type 2
<a href="#">Details</a>	12	84	hsa-miR-770-5p	<a href="#">DHTKD1</a>	dehydrogenase E1 and transketolase domain containing 1



# Stathmin phosphorylation pathway



# AZT AND PROBABLY OTHER NAS

DNA damage

Downregulation miR-770-5p

Upregulation of STMN-1

Tubulin depolymerization

Centrosomal amplification

Chromosomal instability

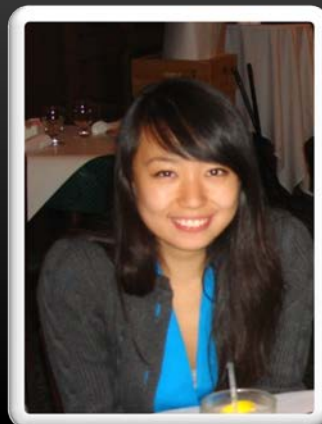




*Lorangelly Rivera-Torres*



*Sayeh Gorjifard*



*Mia Yu*



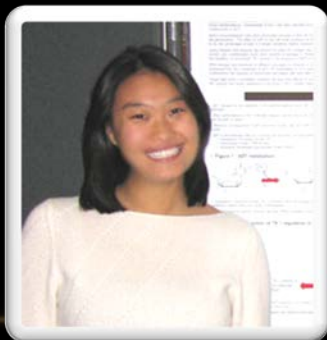
*Andrea Rivera-Rodriguez*



*Jennifer Borojerdi*



*Vanesa Sanchez*



*Jessica Ming*



*Miriam Poirier*