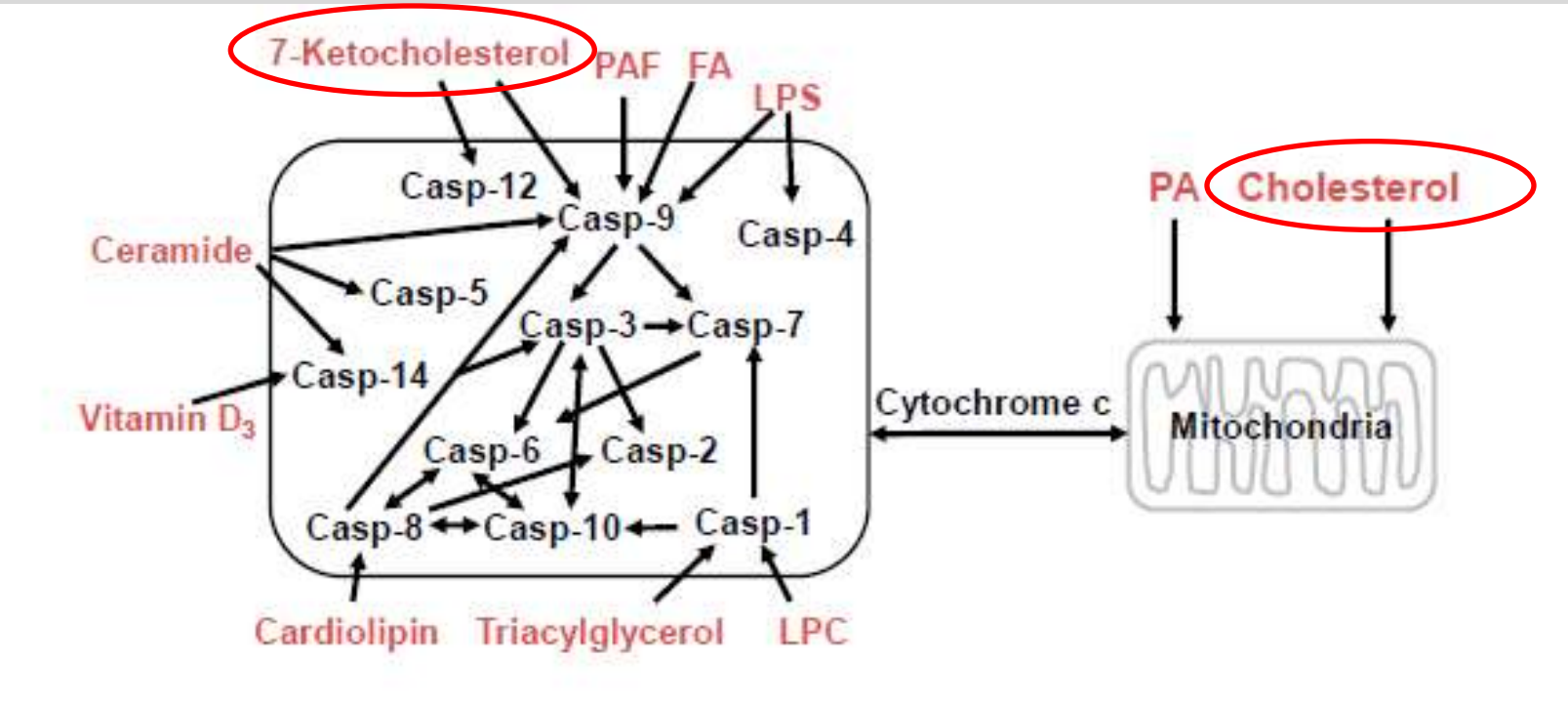
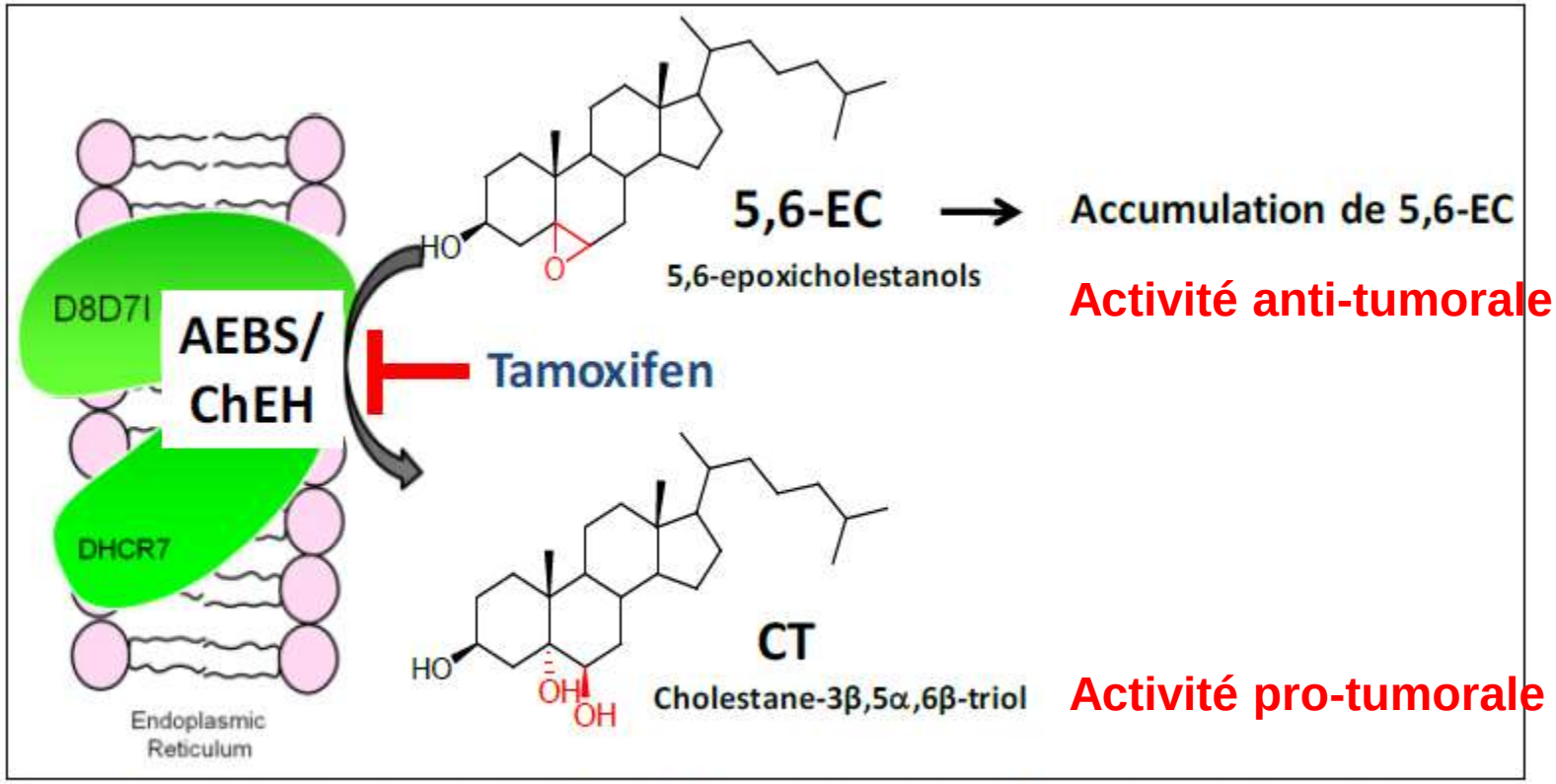
# Implication du cholestérol et de ses dérivés oxydés (7-cétocholestérol) dans la cancérogénèse




**Le cholestérol et les oxystérols sont impliqués dans la régulation de la signalisation apoptotique (activités anti-tumorales)**

Huang C & Freter C Int. J. Mol. Sci. 2015, 16, 924-949

# Tamoxifen et cancer du sein

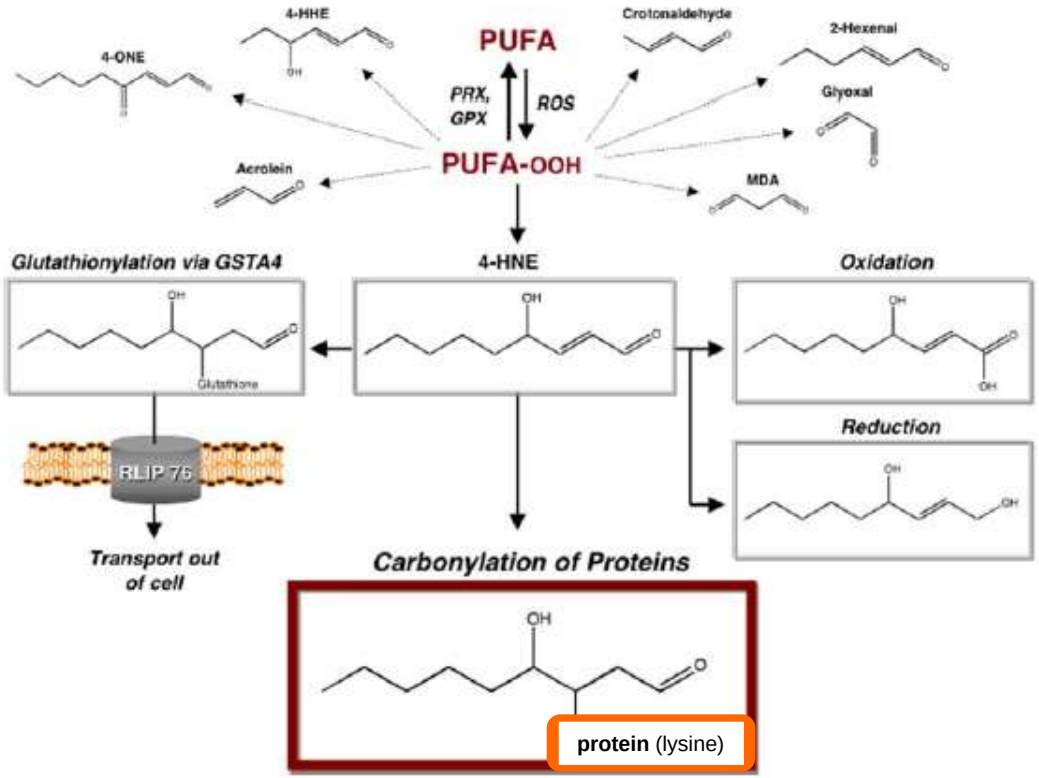



**Le site AEBS porte l'activité ChEH .** En absence de ligands, les 5,6-EC sont hydrolysés en CT. Lorsqu'un ligand, comme le Tam vient se fixer, il provoque l'inhibition de la ChEH et l'accumulation des 5,6-EC.

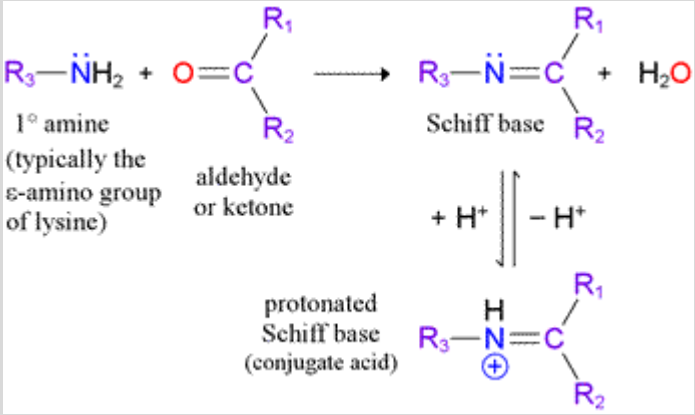
**D8D71:** 3β-hydroxystérol-Δ8-Δ7-isomérase
**DHCR7:** 3β-hydroxystérol- Δ7-réductase
**AEBS:** anti-estrogen binding site
ChEH: cholesterol ester hydrolase

Thèse de Doctorat 2015 – M. Voisin, Univ. Toulouse

# Oxydation des acides gras et carbonylation protéique




FI( ular fates of $\alpha,\beta$-unsaturated aldehydes and carbonylated proteins. ROS stimulate peroxidation of polyunsaturated fatty acids (*PUFA*), an oxidative event that is reversible through reduction by peroxiredoxin (*PRX*) and glutathione peroxidase (*GPX*) enzymes. The lipid hydroperoxides (*PUFA-OOH*) generated are unstable and lead to a variety of reactive aldehydes. The lipid peroxidation products generated include the $\alpha,\beta$-unsaturated aldehydes 4-HNE, 4-ONE, 4-hydroxy-(2*E*)-hexanal (*4-HHE*), (2*E*)-hexenal, crotonaldehyde, and acrolein as well as the dialdehydes glyoxal and malondialdehyde (*MDA*). GSTA4 catalyzes the conjugation of the highly reactive $\alpha,\beta$-unsaturated aldehydes to glutathione, leading to their efflux from the cell by the glutathione conjugate transporter RLIP76. In addition, oxidation by aldehyde dehydrogenase or reduction by alcohol dehydrogenase, aldehyde reductase, or aldose reductase converts free aldehydes into less toxic molecules. The $\alpha,\beta$-unsaturated aldehydes that escape cellular metabolism serve as electrophiles in the covalent modification of proteins via non-enzymatic Michael addition. The resulting aliphatic carbonyl adducts on cysteine, histidine, or lysine residues may alter the activity of protein targets or cause them to become degraded by the proteasome.




*Grimsrud PA et al. Oxidative stress and covalent modification of protein with bioactive aldehydes. J Biol Chem;283(32):21837-41.*

Gérard LIZARD : EA7270
Univ. Bourgogne Franche Comté / INSERM

# AVAILABLE METHODS ALLOWING THE CHARACTERIZATION OF APOPTOSIS, NECROSIS / NECROPOTOSIS, AND AUTOPHAGY

*Gérard Lizard,*

# FUNCTIONNAL CRITERIA ASSOCIATED WITH APOPTOSIS, NECROSIS, AND NECROPTOSIS

- **Microscopy, flow cytometry, biochemistry**

**- enhanced permeability of cytoplasmic membrane (trypan blue, fluorescent probes, LDH)**

**- externalization of phosphatidylserine**

double staining with AnnexinV /propidium iodide (PI) (or aminoactinomycine D (AAD)) to distinguish between normal (AnnexinV$^-$/PI $^-$), necrotic (AnnexinV$^+$/PI$^+$), and apoptotic (AnnexinV$^+$/PI$^-$) cells

**- Coloration SYTO16 - IP**

**- Sub-G1 peak (not present in normal and necrotic cells)**

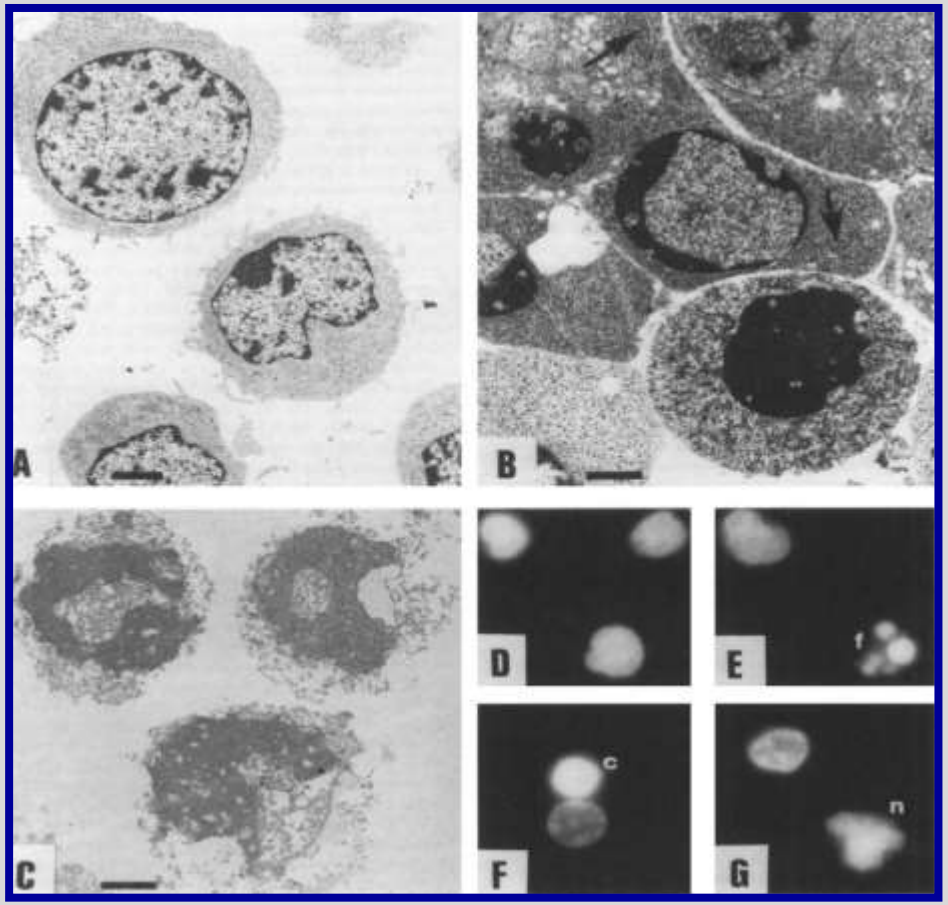
**- loss of transmembrane mitochondrial potential:** numerous fluorescent probes available. Currently, Dio$C_6$(3) and JC1 are the most reliable.

**- FLICA (fluorochromes labeled inhibitors of caspases):** *in situ* identification of activated caspases (does not permit to distinguish between necrosis and caspase-independent cell death)

**- TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling):** *in situ* identification of internucleosomal DNA fragmentation

*Gérard Lizard,*

# APOPTOSIS / NECROSIS: MORPHOLOGICAL CRITERIA




**Electron microscopy** A: Control. B: Cells treated by VP-16; apoptotic cells with condensed and perinuclear chromatin are observed, cytoplasmic and nuclear membrane integrity are preserved as well as morphology of mitochondria (arrow). C: Cells treated by NaN3; necrotic cells are characterized by a loss of integrity of cytoplasmic and nuclear membranes, degradation of cytoplasm and chromatin.

**Fluorescence microscopy** after staining with Hoechst 33342 D: Control cells. Nuclei show regular contours. E: Cells treated by VP-16; cell with fragmented (f) nucleus. F: Cells treated by VP-16; cell with condensed (c) nucleus. G: Necrotic (n) cells observed under treatment by NaN3;
the nucleus is diffuse and irregular. (***Lizard G et al. Cytometry 1995, 21: 275-283***)

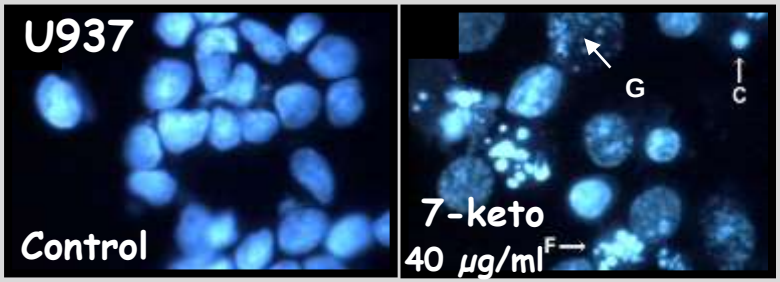
*Gérard Lizard,*

# MORPHOLOGICAL CRITERIA ASSOCIATED WITH APOPTOSIS, NECROSIS AND NECROPTOSIS

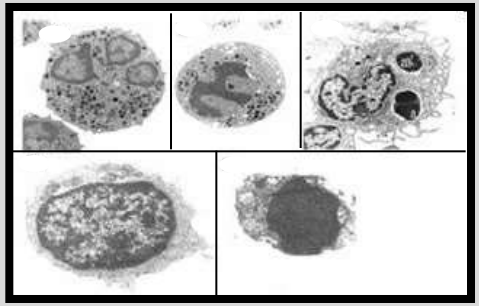
*** Phase contrast microscopy: loss of refringence; loss or not of cell adhesion?**

*** Brightfield microscopy: after staining with GIEMSA,…**

*** Fluorescence microscopy: Hoechst staining allows to easily distinguish between normal, necrotic and apoptotic cells (nuclear criteria)**



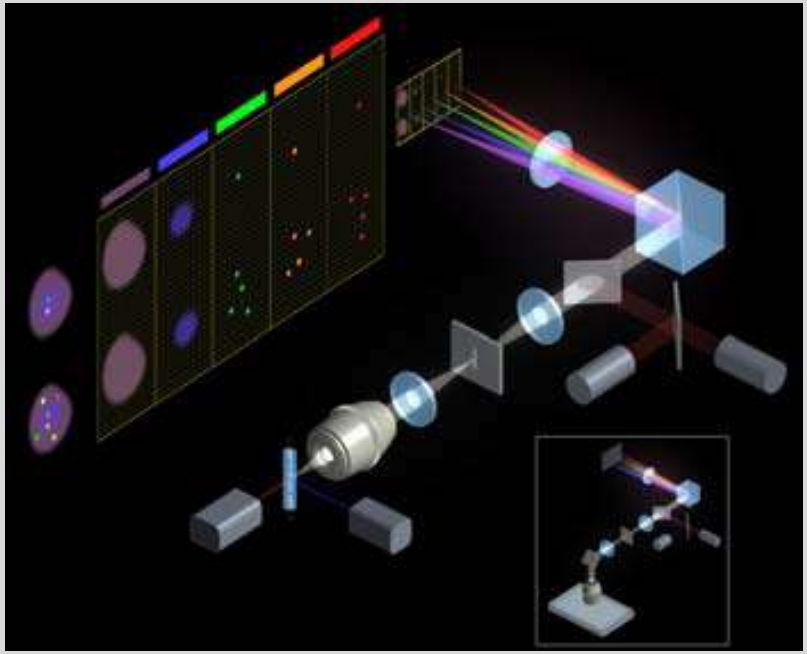

*** Transmission electron microscopy (nuclear criteria)**



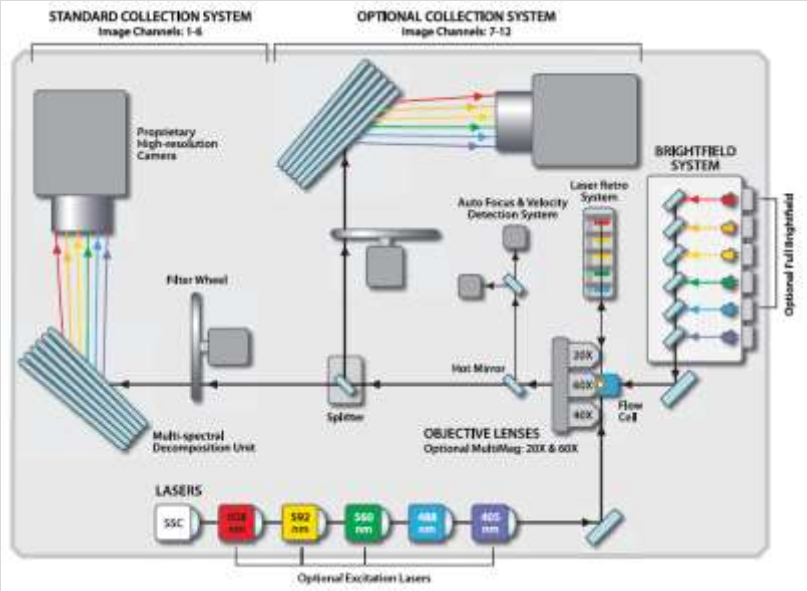
*** Flow cytometry: changes of light scatter properties *on non-fixed cells* (FSC ↓ ; SSC more or less ↑ )**

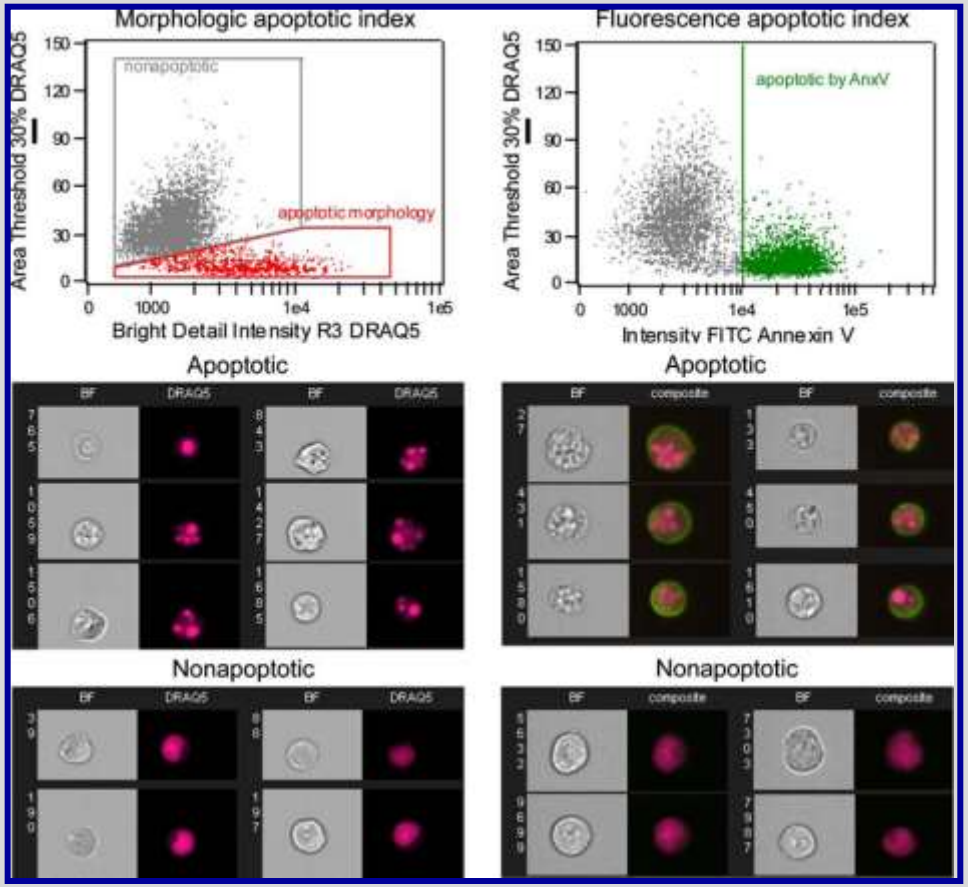
*Gérard Lizard,*

# Annexin V-FITC / PI (Amnis technology)



*http://www.amnis.com*

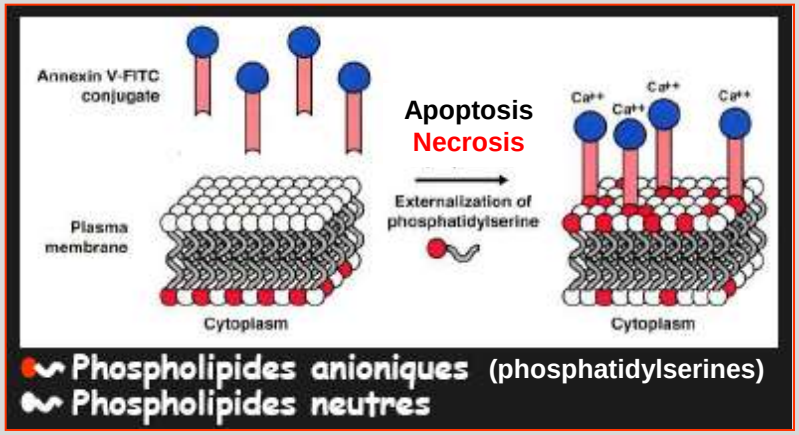


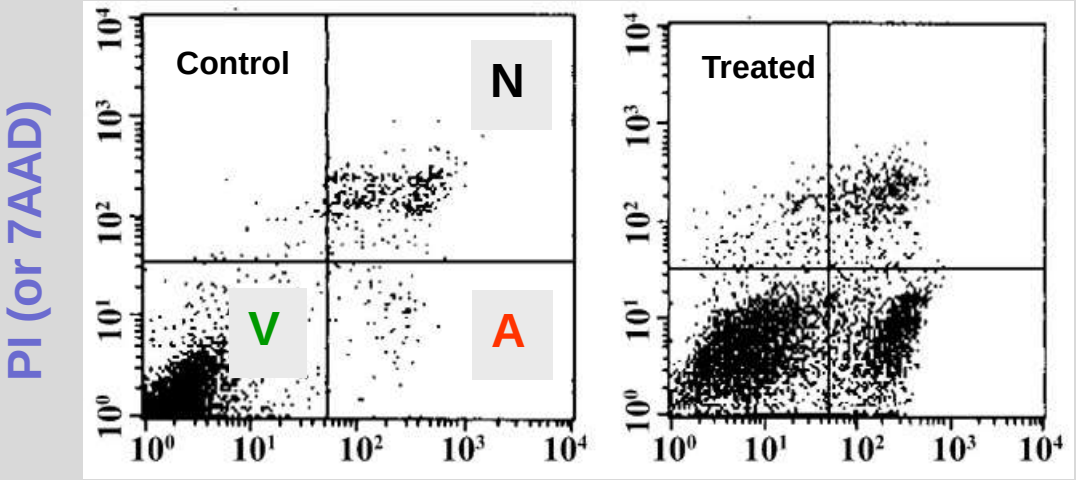

*Henery S et al. Apoptosis 2008, 13: 1054-1063*

*Gérard Lizard,*

# Phosphatidylserine externalization : Annexin V-FITC / PI test




| Cells | Annexine V | PI |
|---|---|---|
| Viable (**V**) | − | − |
| Apoptotic (**A**) | + | − |
| Necrotic (**N**) | + | + |




**PI: propidium iodide**
**7 AAD: 7 amino actinomycin D**

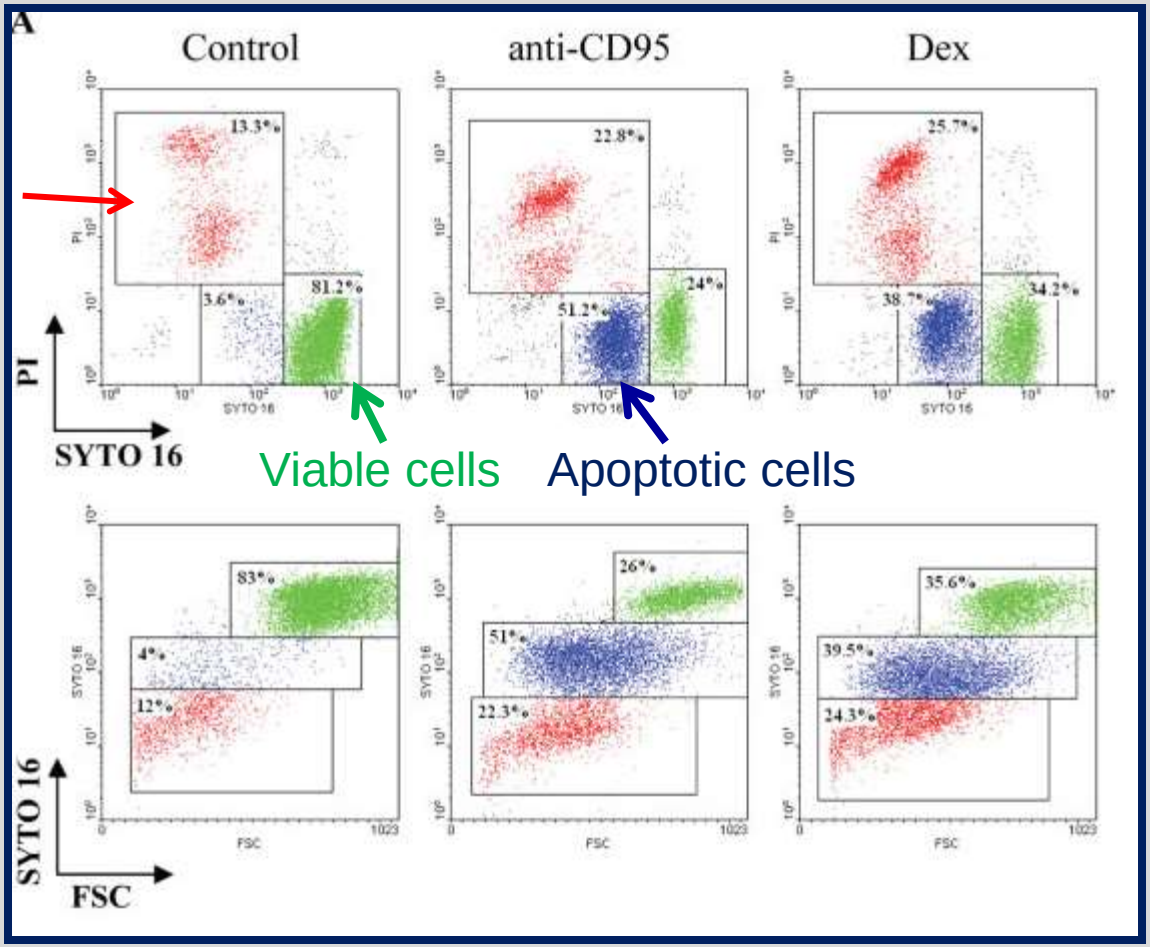
*Gérard Lizard,*

# Towards an Understanding of Apoptosis Detection by SYTO Dyes

Donald Wlodkowic,1* Joanna Skommer,1 and Jukka Pelkonen1,2
1Institute of Clinical Sciences, Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland
2Department of Clinical Microbiology, Kuopio University Hospital, Kuopio, Finland

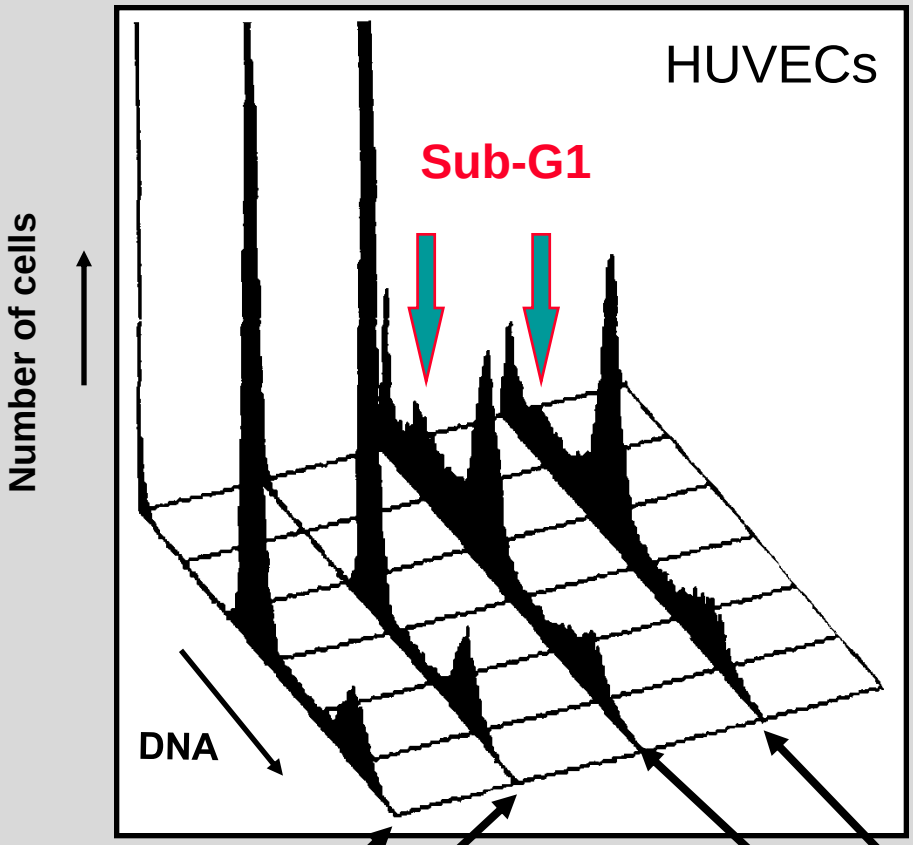
**Cytometry Part A** 71A:61–72 (2007)

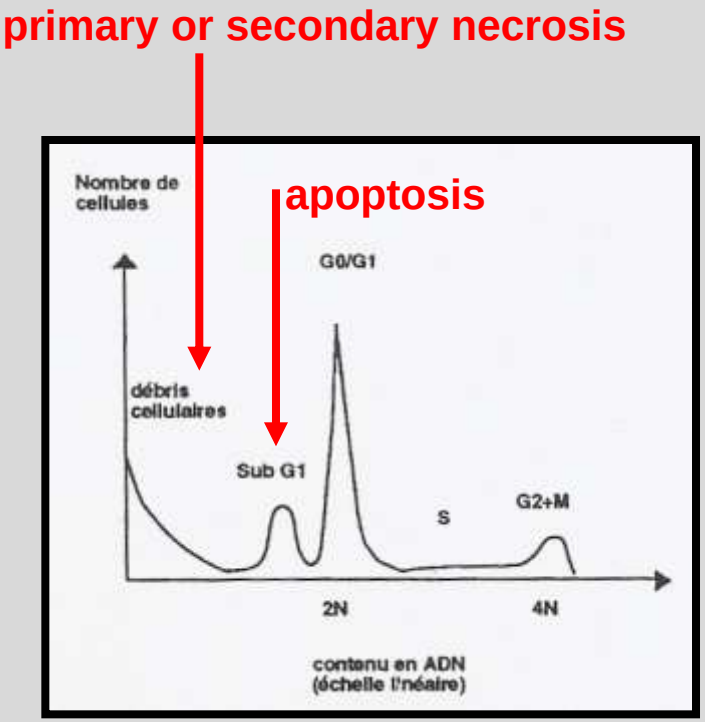
Necrotic cells




*Gérard Lizard,*

# Sub-G1 (FCM)




control

cholesterol

7-ketocholesterol

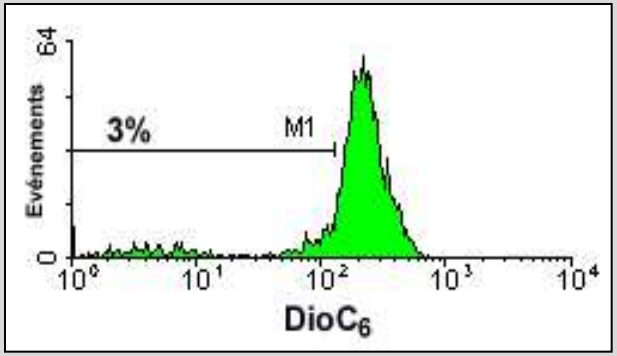
7ß-hydroxycholesterol

*Lizard G et al. Am J Pathol 1996, 148: 1625-1638*

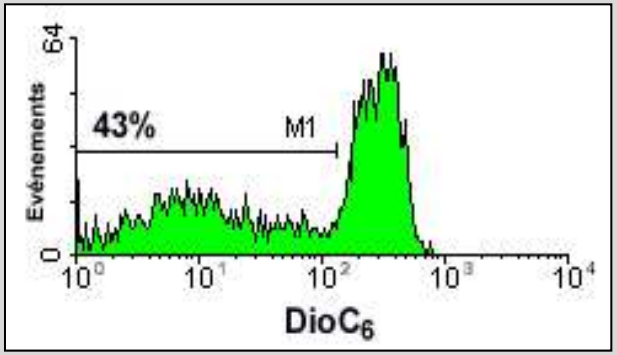
*Gérard Lizard,*

# Mitochondrial potential (FCM)

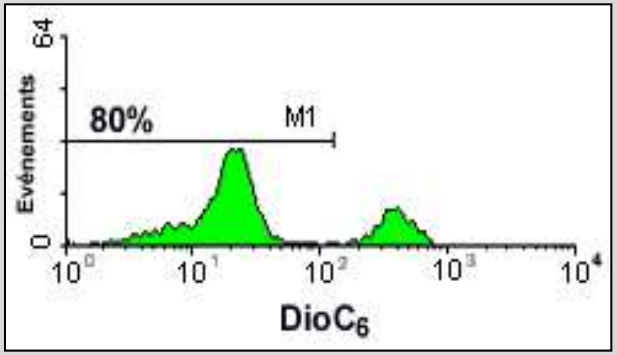
## Control

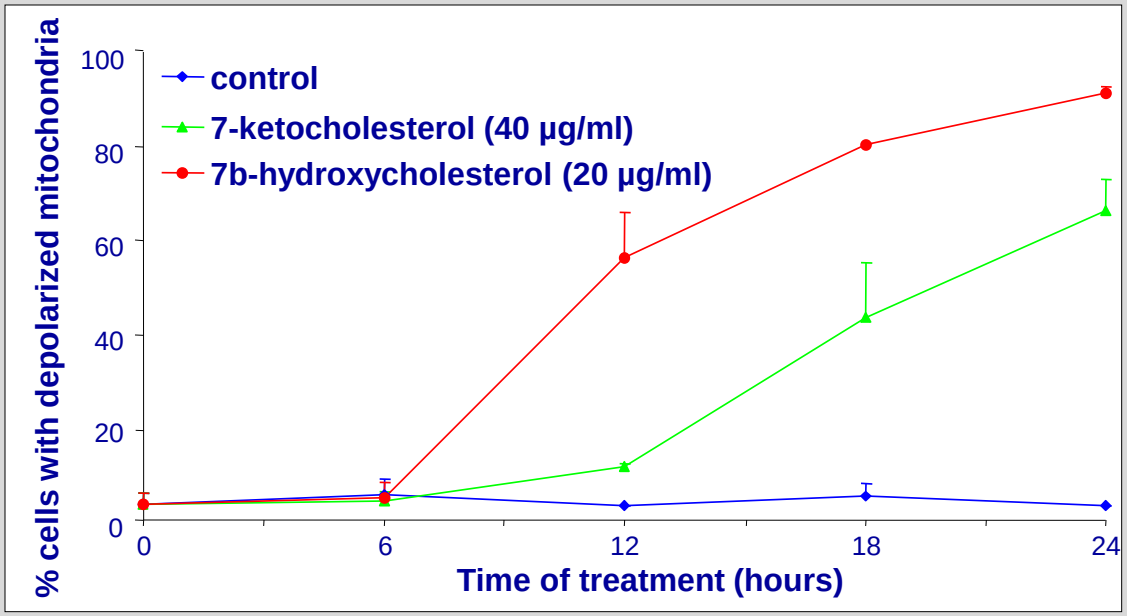



## 7-ketocholesterol



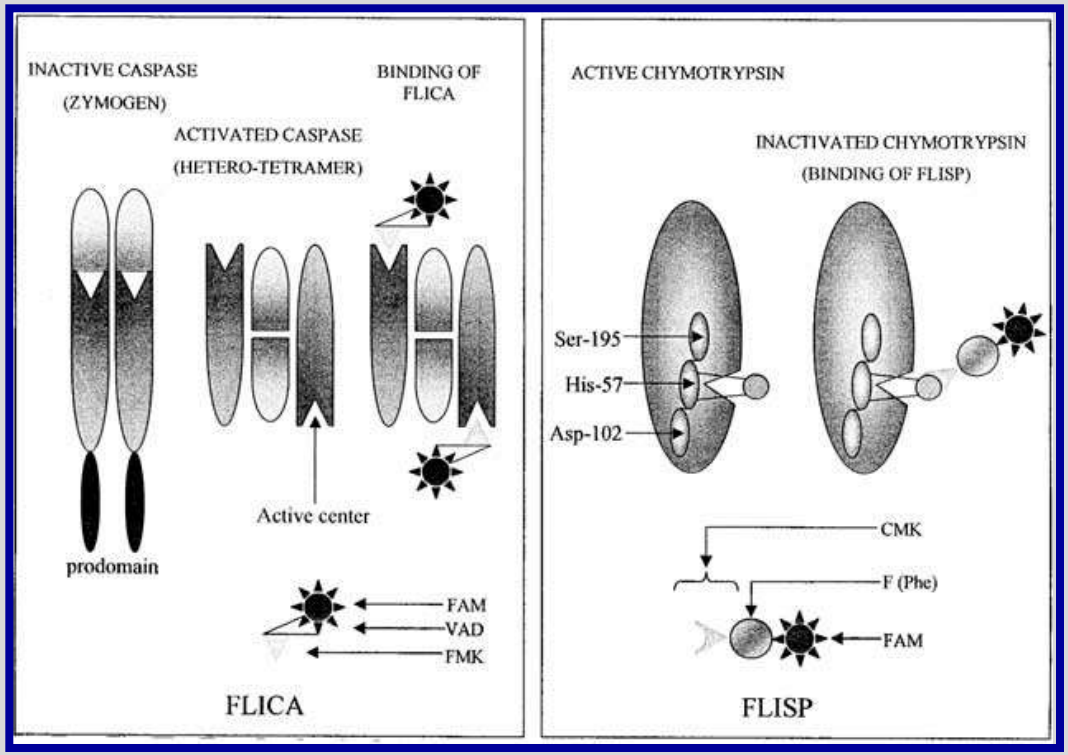

## 7β-hydroxycholesterol



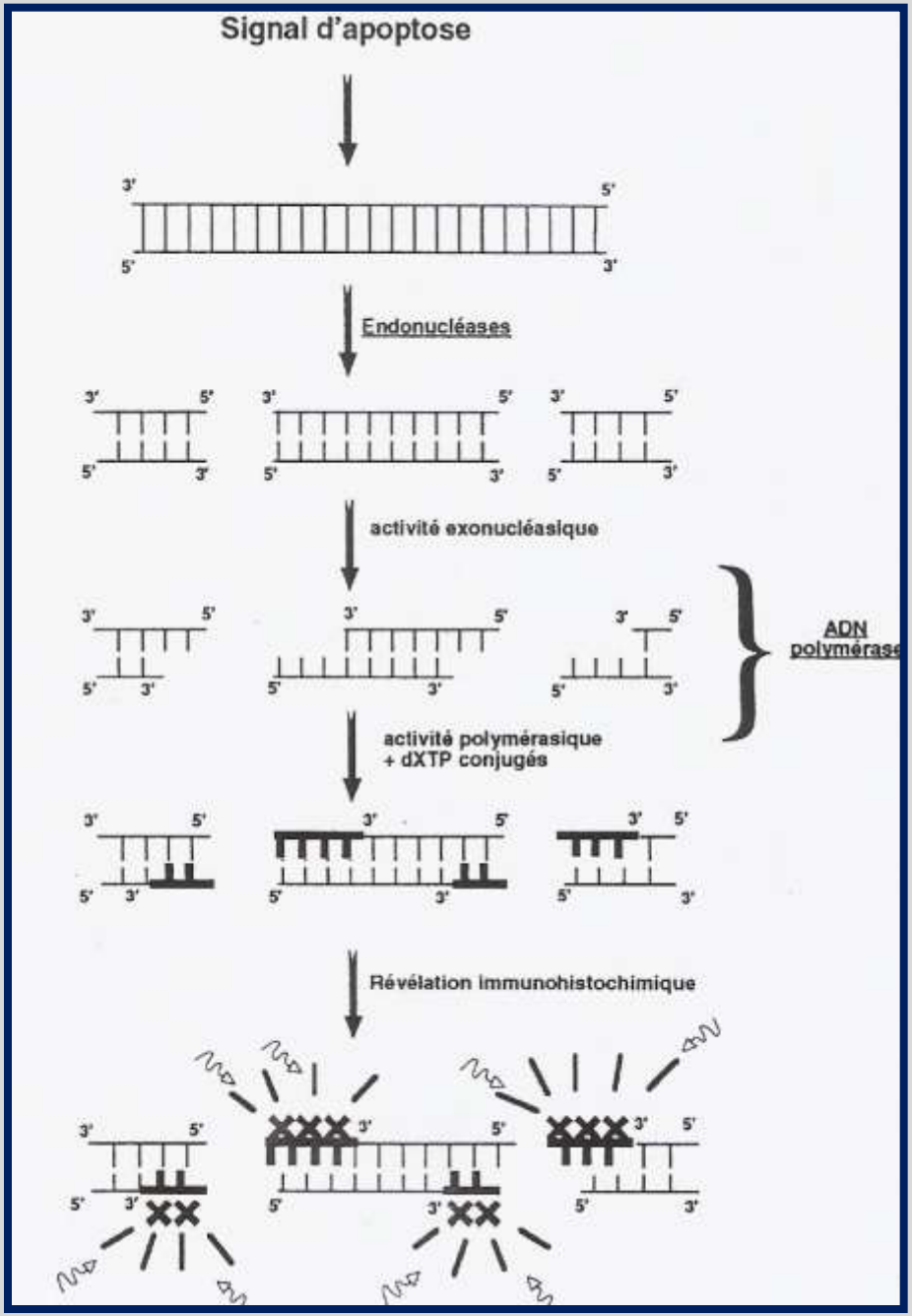

*Gérard Lizard,*

# FLICA / FLISP (Microscopy, Flow Cytometry)

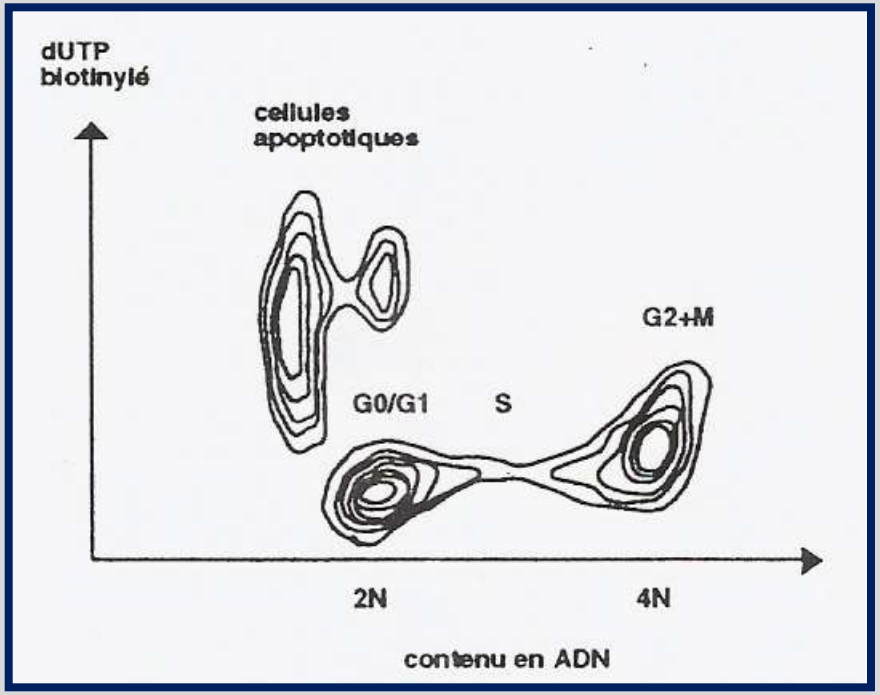



*Grabarek J & Darzynkiewicz Z Exp Hematol 2002, 30: 982-989*

*Gérard Lizard,*

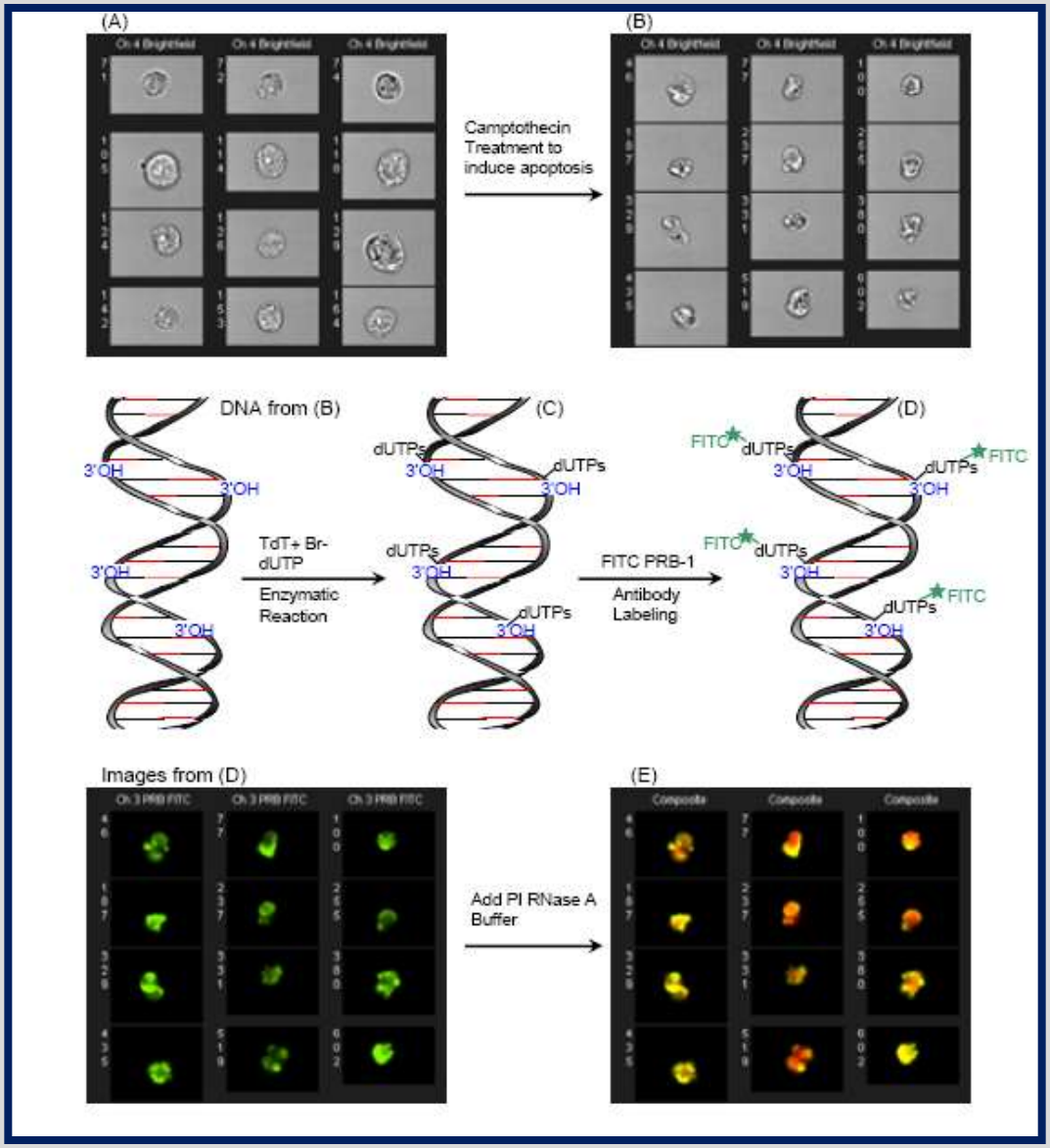



# TUNEL (Flow Cytometry)



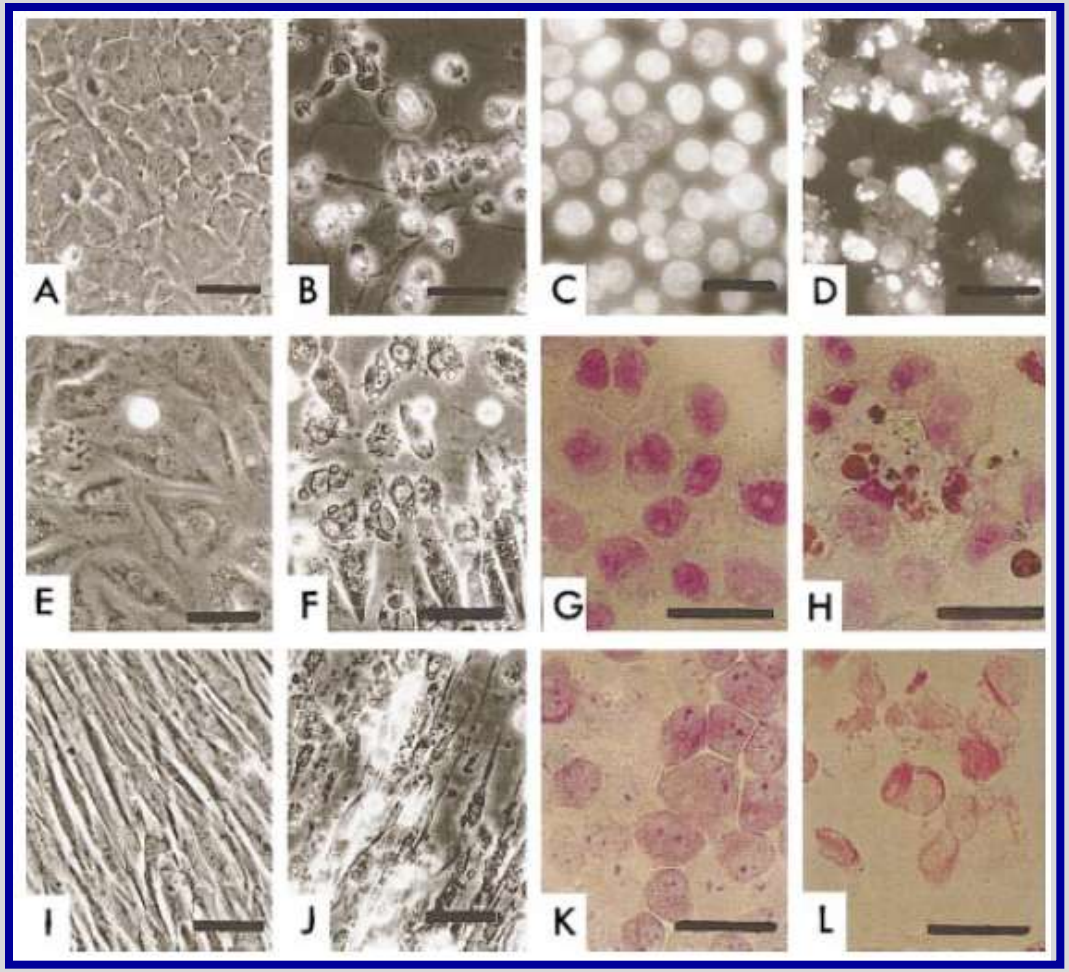

*Gérard Lizard,*

# TUNEL
# (Amnis technology)




*Gérard Lizard,*

# Phase contrast microscopy, Hoechst 33342, GIEMSA, TUNEL




**Apoptosis = TUNEL positive cells**
**Necrosis = TUNEL negative cells**

***Lizard G et al., Arterioscler Thromb Vasc Biol 1999, 19: 1190-2000***

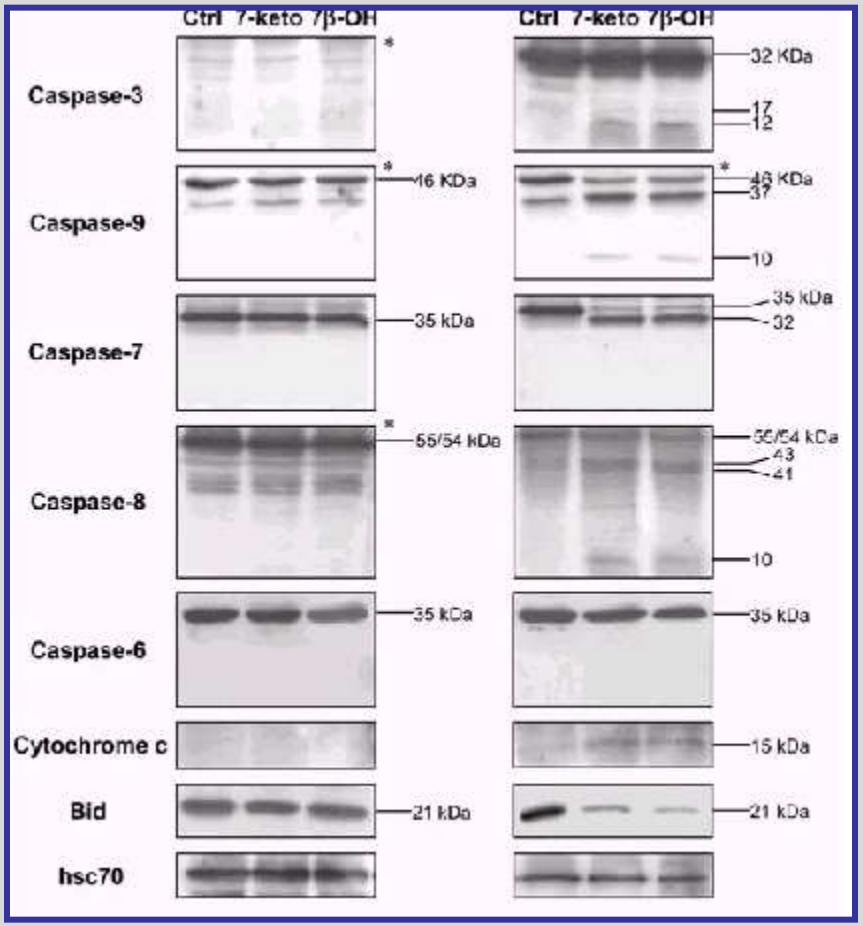
*Gérard Lizard,*

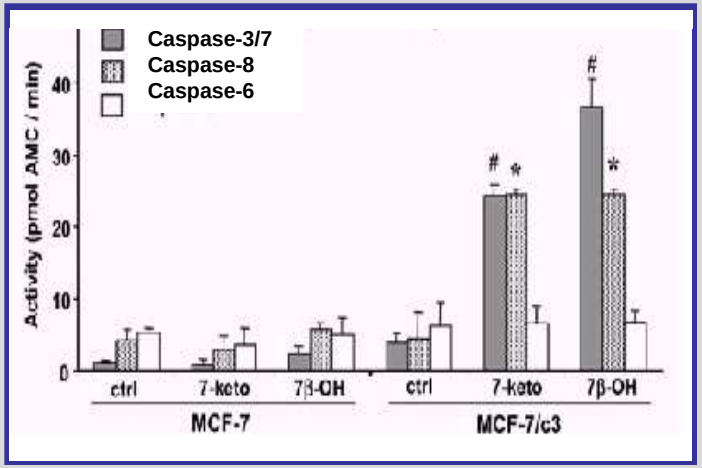
# BIOCHEMICAL CRITERIA ASSOCIATED WITH APOPTOSIS, NECROSIS, AND NECROPTOSIS

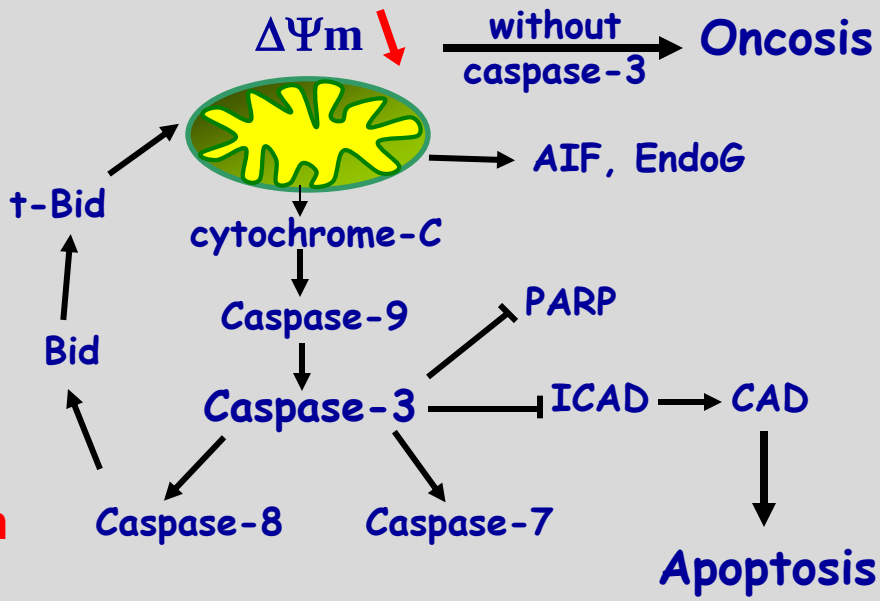
* Analysis of the DNA fragmentation pattern on agarose gel
  - Apoptosis: DNA ladder, multiple of 150-200 base pairs (internucleosomal DNA fragmentation)
  - Necrosis: no DNA ladder, smears

* Enzymatic activities (LDH)

* Electrophoresis on acrylamide gel and Western Blotting



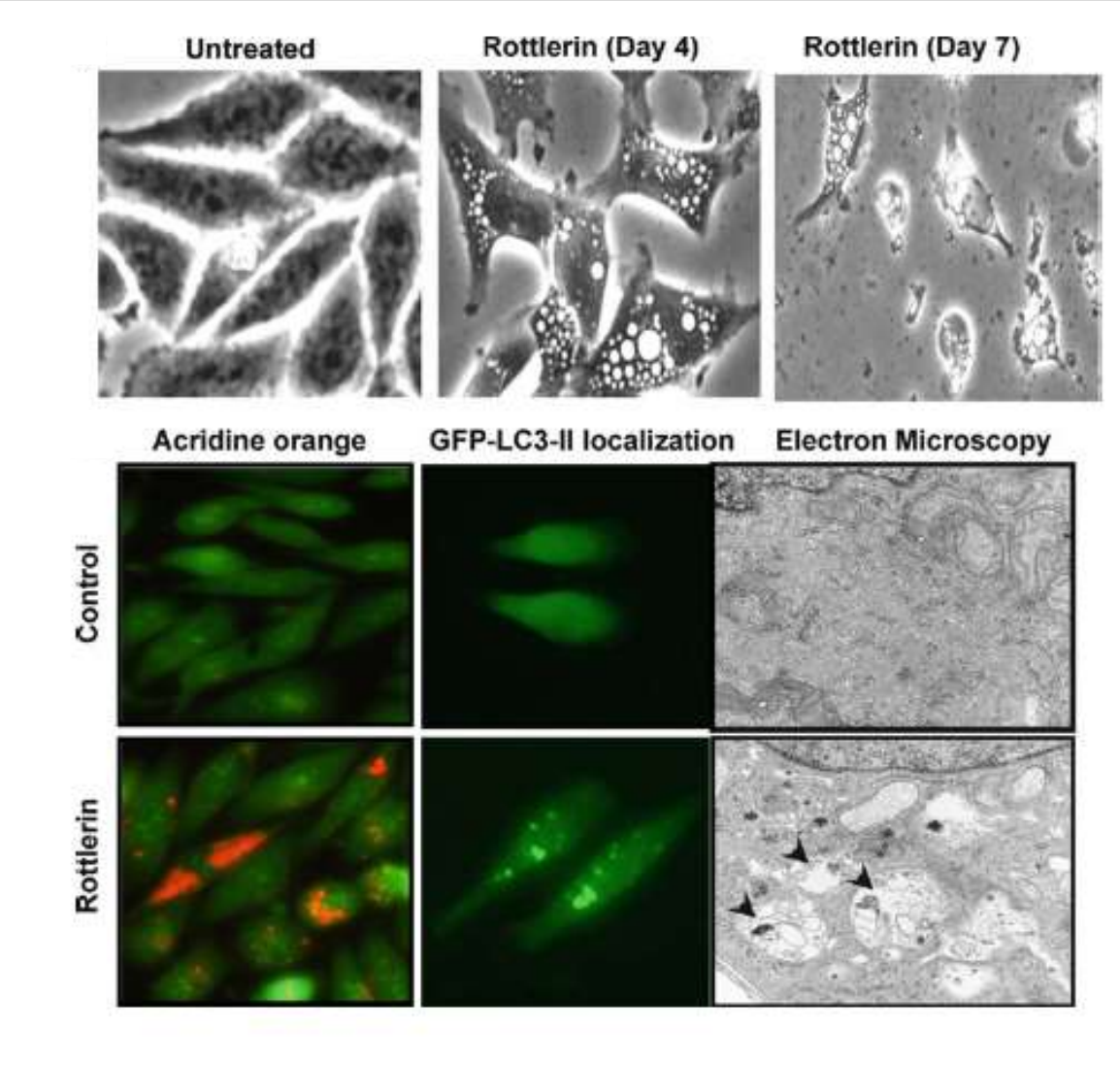




**Necrosis/Oncosis = no caspase activation**

*Prunet C et al. Cell Death Differ, 2001; J Biochem Mol Toxicol, 2005*

*Gérard Lizard,*

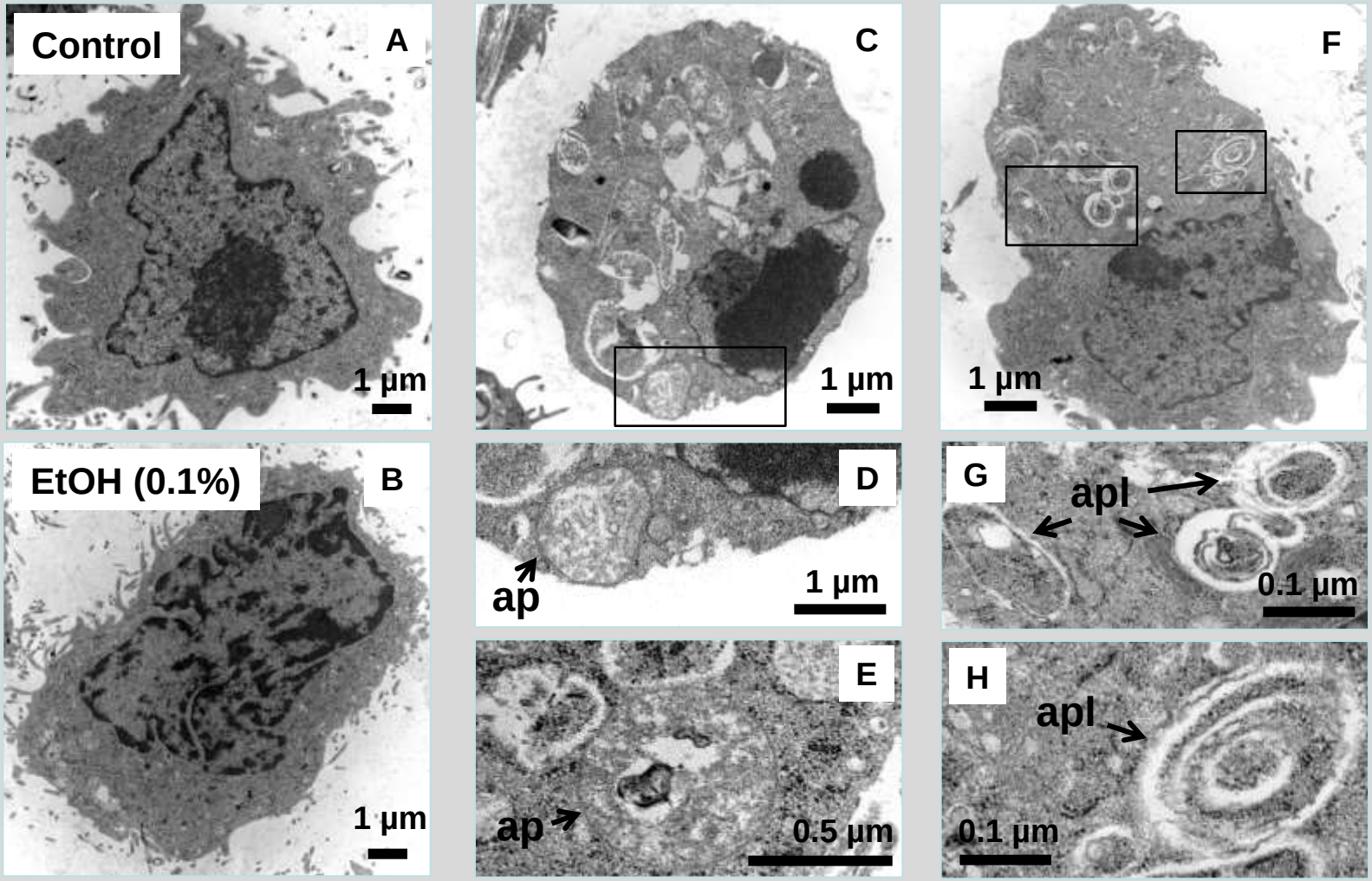
# CHARACTERIZATION OF AUTOPHAGY: 'VISUAL' CRITERIA




*Gérard Lizard,*

# CHARACTERIZATION OF AUTOPHAGY:
# ULTRASTRUCTURAL CRITERIA – Transmission Electron Microscopy

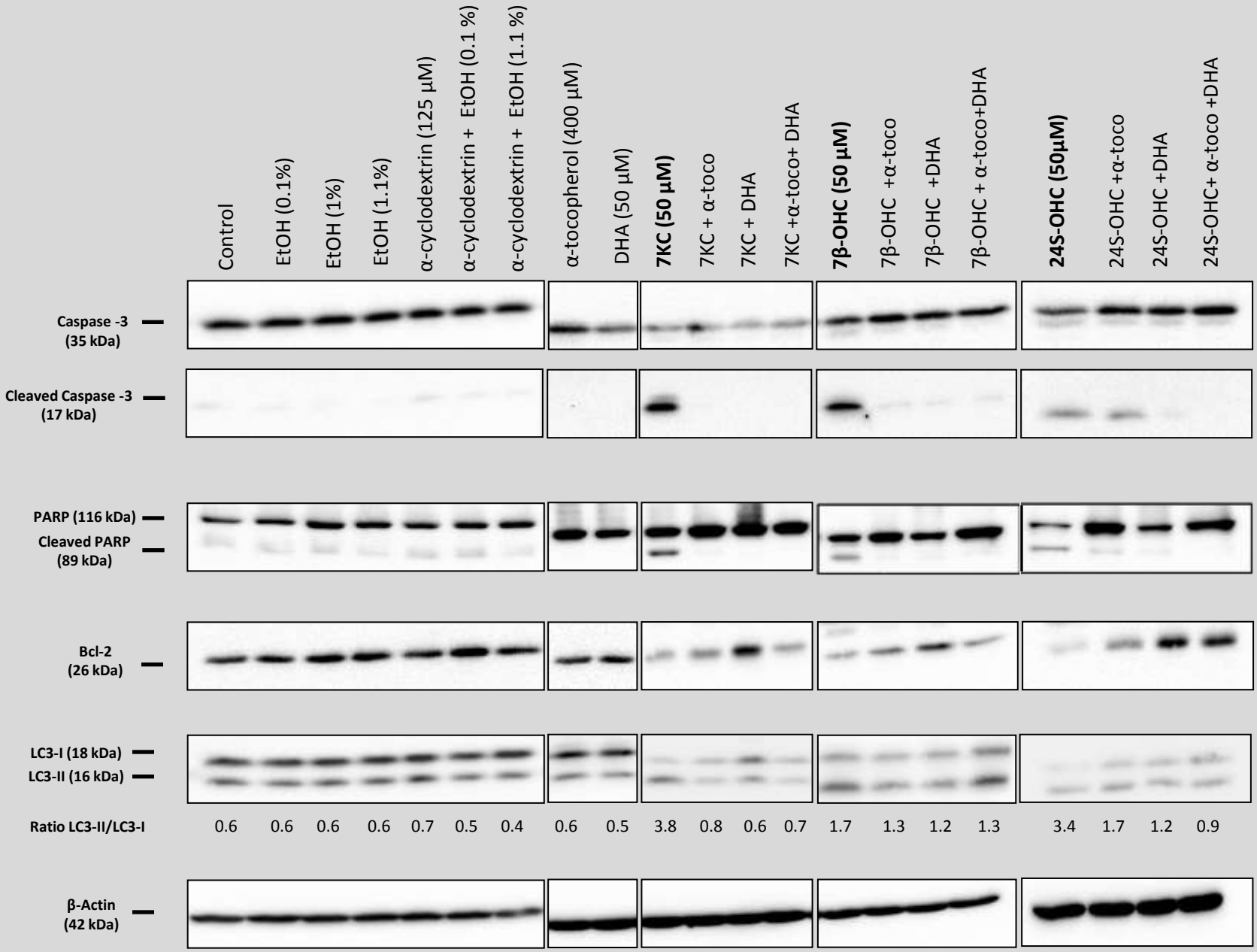
**7KC (50 µM, 24 h)**



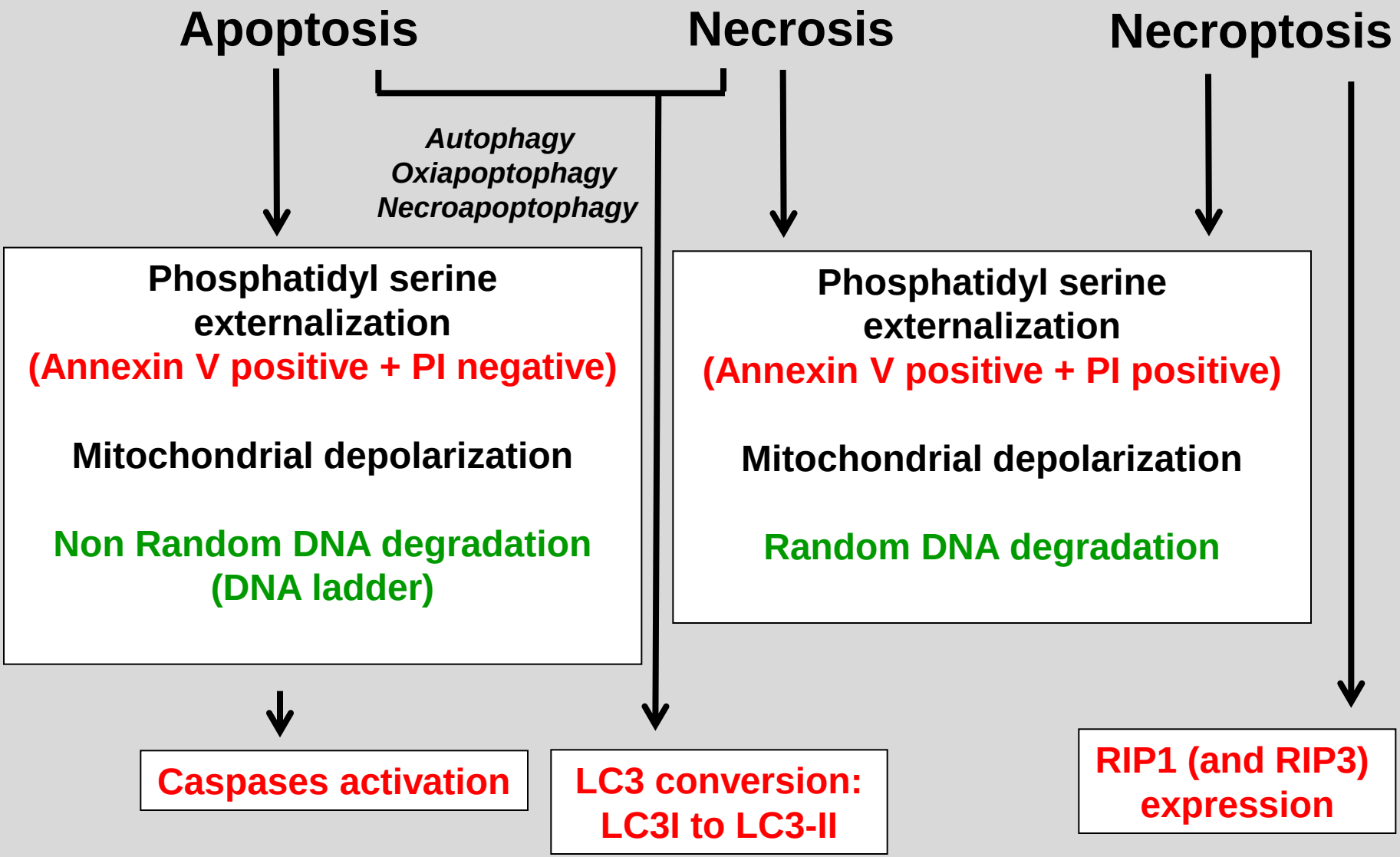

**ap**: autophagosome; **apl**: autophagolysosome

*Gérard Lizard*

# CHARACTERIZATION OF AUTOPHAGY: BIOCHEMICAL CRITERIA
## Murine oligodendrocytes (158N) - autophagy and apoptosis (oxiapoptophagy)




*Gérard Lizard,*




*Gérard Lizard,*