Programa Cooperación Farma-Biotech

Jornada II: **Oncología**

Minerval®:
Treatment of glioma and other types of cancer

LIPOPHARMA
Lipid Science for life

Vicenç Tur. CEO, Co-founder

Barcelona, 13 de abril de 2011

CataloniaBIO
Associació catalana d'empreses de biotecnologia

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

farmaindustria

Programa Cooperación Farma-Biotech

Jornada II: **Oncología**

Content

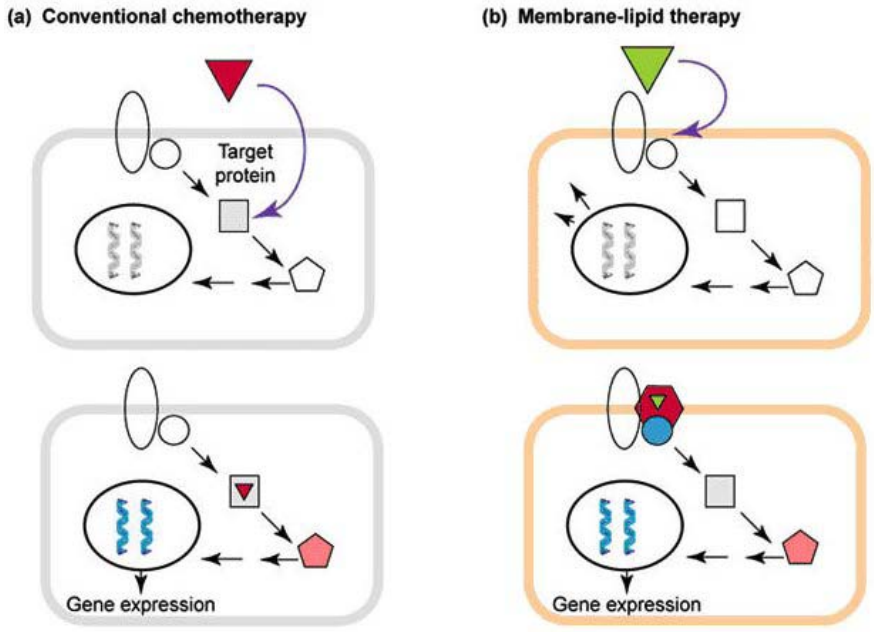
1. **The Company**
2. **The Technology platform: TLM**
3. **The Product**
   a) Therapeutic focus
   b) Innovative mechanisms of action
   c) Differential features facing the market
   d) Current status of development
   e) IPR protection
   f) Pitfalls & Risks to be considered
4. **Availability for cooperation**

Minerval® for the treatment of glioma

1. Based on an **innovative Technology platform**: Membrane Lipid Therapy (MLT)
2. **Novel** & **well elucidated** MOA: *Ras/MAP Kinase pathway inhibition*
3. High **specificity**, very high **efficacy** (in cells & animals) & **non toxic**
4. Ready to move into **clinical development** (2H 2011)

Lipopharma [2007] is an pioneering **science-driven** biopharmaceutical company based in Palma de Mallorca (Spain) that focuses on the discovery, rational design and initial clinical development of next generation medicines on the basis of a new therapeutic approach: the **Membrane Lipid Therapy** (MLT)

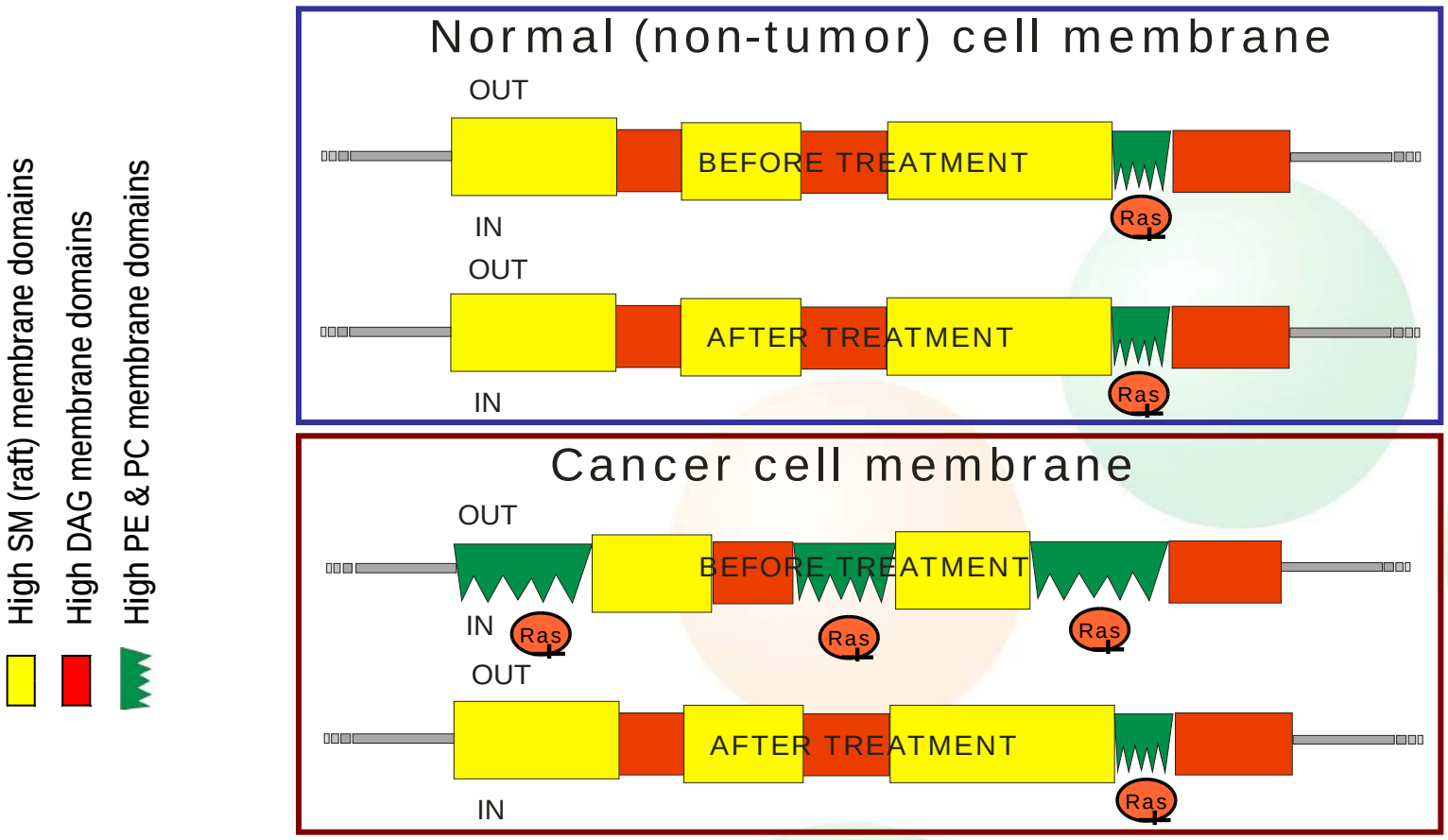
Scientific Background: Membrane Lipid Therapy (TLM)



Escribá (2006) Trends in Molecular Medicine 12:34-43

Minerval® was designed on the basis of MLT, a disrupting innovative therapeutic approach consisting in the design of molecules that, instead of directly targeting intracellular proteins (as happens with most current drugs), they interact with **membrane lipids**, regulating its composition and the structures they form, and therefore regulating also the molecular signaling pathways involving peripheral proteins and downstream events. MLT drugs influence lipid organization & composition in cell membranes based on **structure–function** principles.

Minerval®: MLT in Action

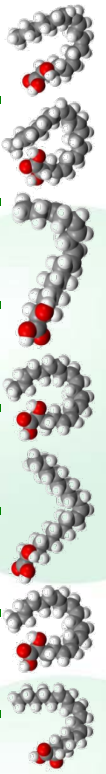


Minerval® induces a rise in the levels of SM (rafts, yellow) and DAG (red) and decreases of PE (green). These changes only affect cancer but not normal cells, causing a specific inactivation of Ras (over-activated in cancer cells) and downstream molecular events (e.g., the MAP kinase pathway) in all cancer cells studied (glioma, lung cancer, leukaemia)

Therapeutic products in development based on the MLT

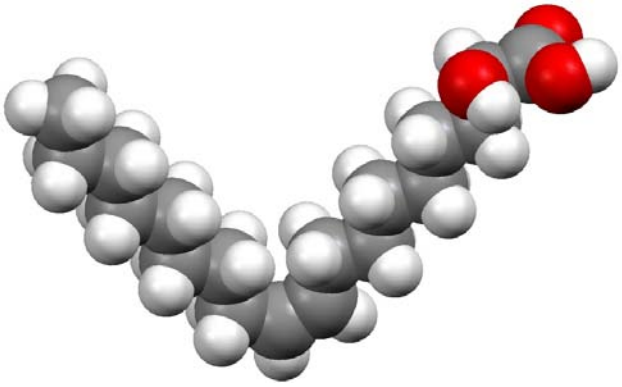
PIPELINE

PRODUCT	THERAPEUTIC AREA	DEVELOPMENT PHASE					
		RESEARCH	PRECLINICAL	PHASE I	PHASE II	PHASE III	REGIST.
Minerval®	Cancer	■	■				
LP226A1	Alzheimer's Disease, CNS disorders	■					
LP204A1	Inflammation	■					
LP10218	Cancer*	■					
LP20104	Cancer*	■					
LPA181	Spinal Cord Injury	■					
LP205A1	CNS, Metabolic & Cardiovascular disorders	■					
LP301T1	Metabolic disorders, Cancer	■					



*out-licensed to AB-Therapeutics

MINERVAL®



MINERVAL® *(2-hydroxyoleic acid) in Oncology*

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Lipid Science for life

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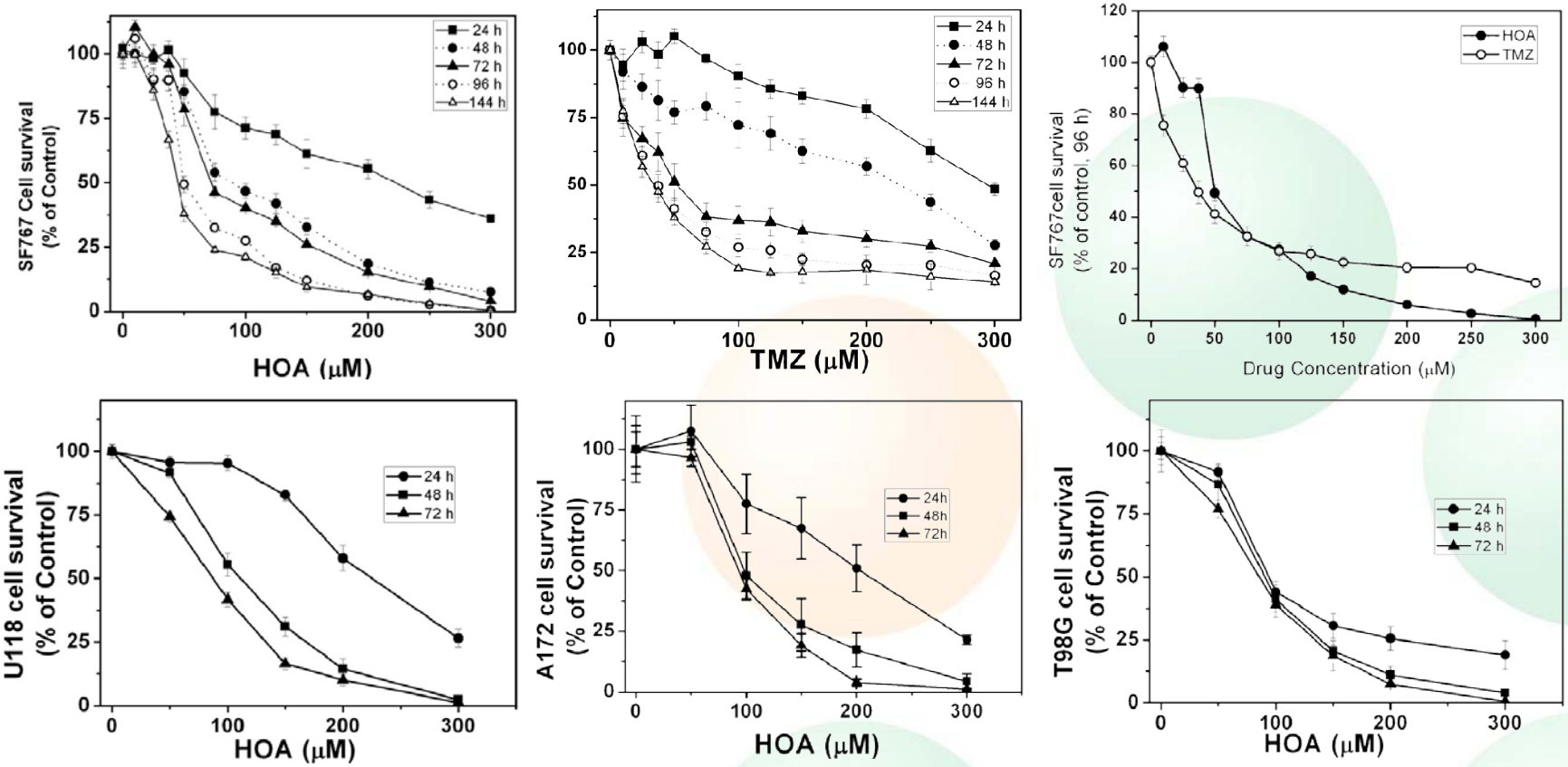
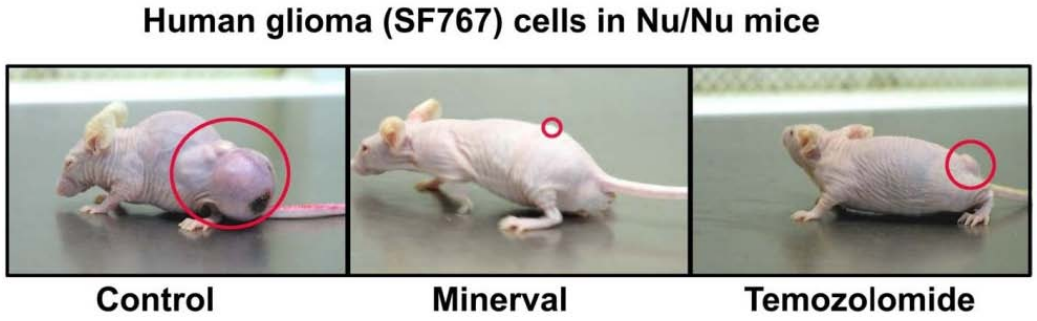
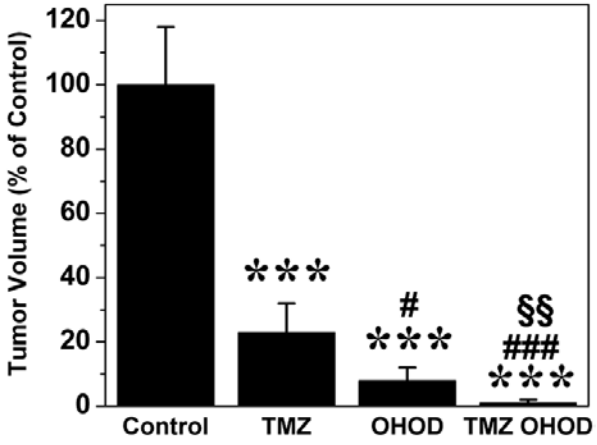
Minerval® exhibits very high efficacy in cellular models ...

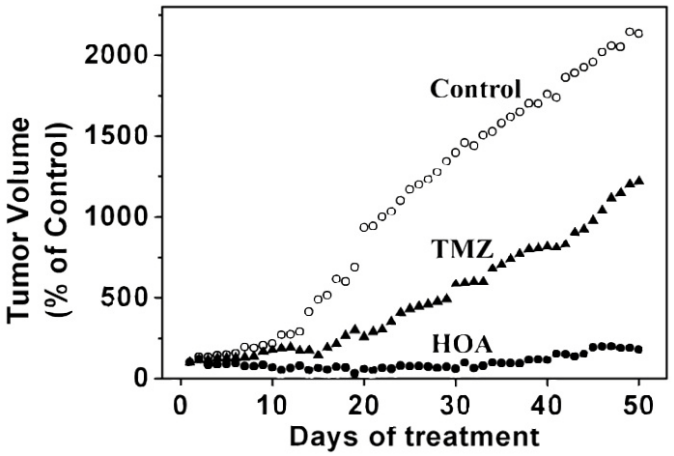


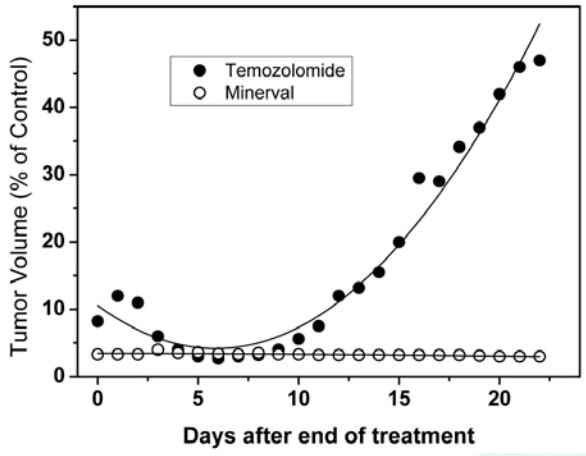
Minerval® (HOA) is able to inhibit, in a time and concentration dependent manner, the growth of several human glioma cell lines (SF767, U118, A172, T98G). In SF767 cell line, Minerval® clearly demonstrates a superior efficacy than temozolomide, which is not able to kill all cancer cells at 300mM

Effect of Minerval® in animal models of human brain tumours (GLIOMA) compared with temozolomide



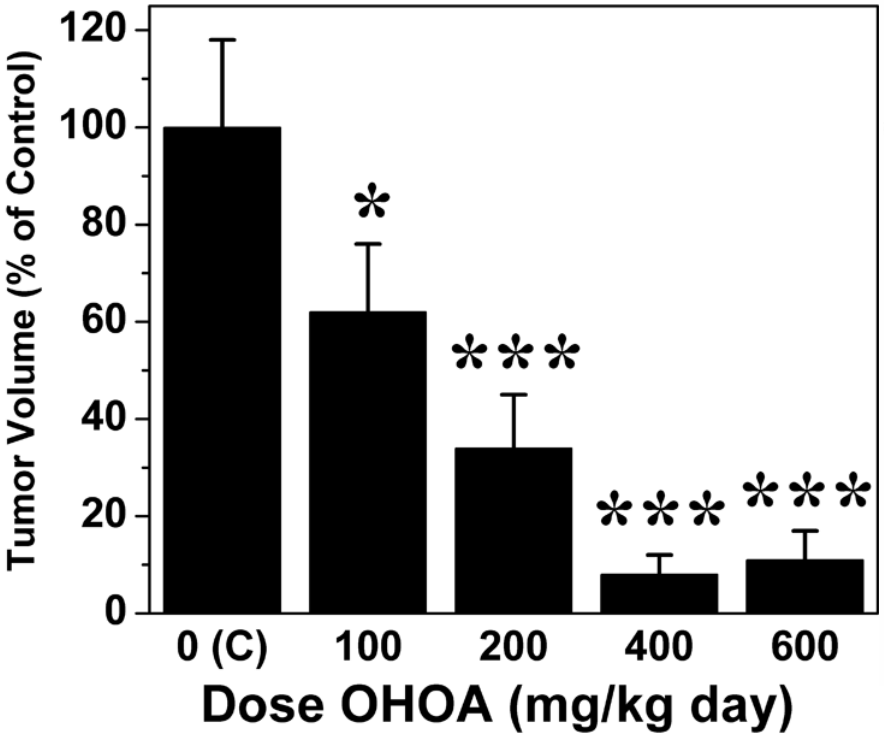




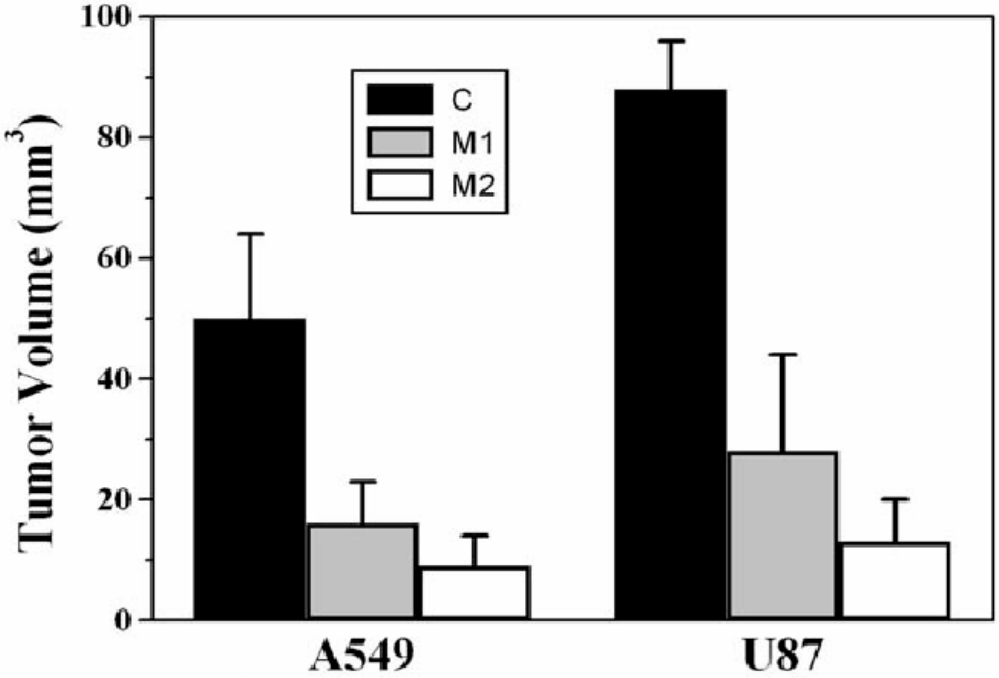


Minerval® has also demonstrated a potent anticancer effect in xenograft animal models, clearly outperforming temozolomide. Moreover, animals treated with Minerval® do not show tumour relapse after treatment termination, as it happens with animals administered with temozolomide (bottom right)

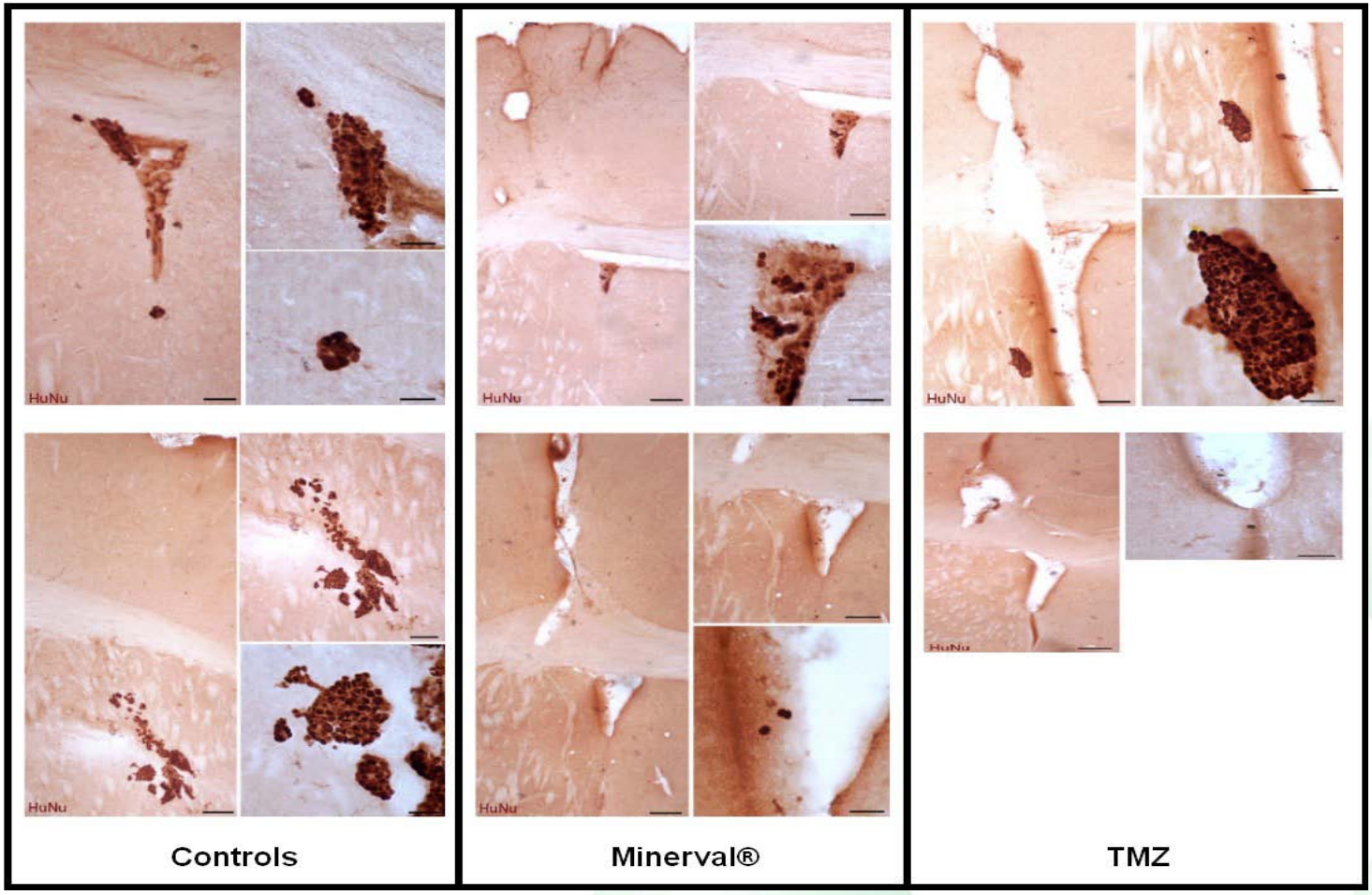
Effect of treatment with Minerval® in LUNG tumours (A549) and in Brain tumours (GLIOMA U87) with 75 (M1) & 125 mg/kg. (M2) against control group (C).



Dose response of Minerval® (OHOA) in GLIOMA tumours (U87)

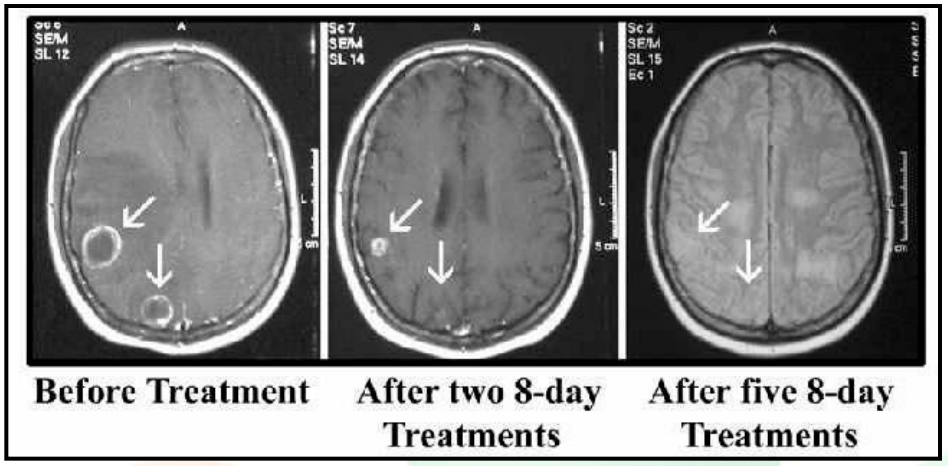


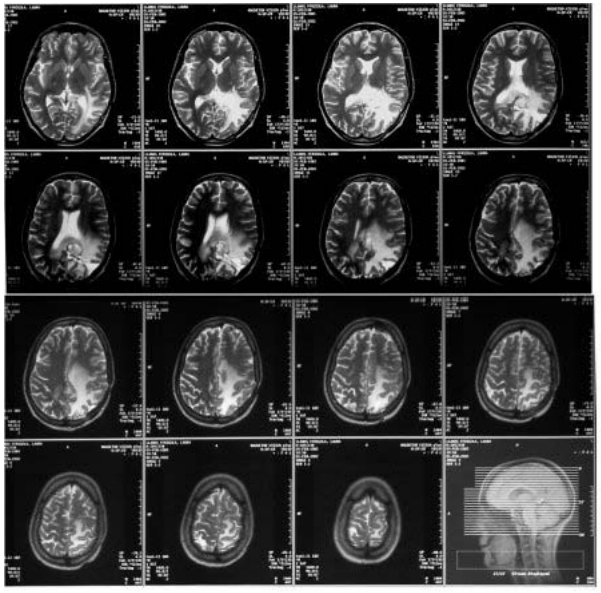
Minerval® crosses the BBR



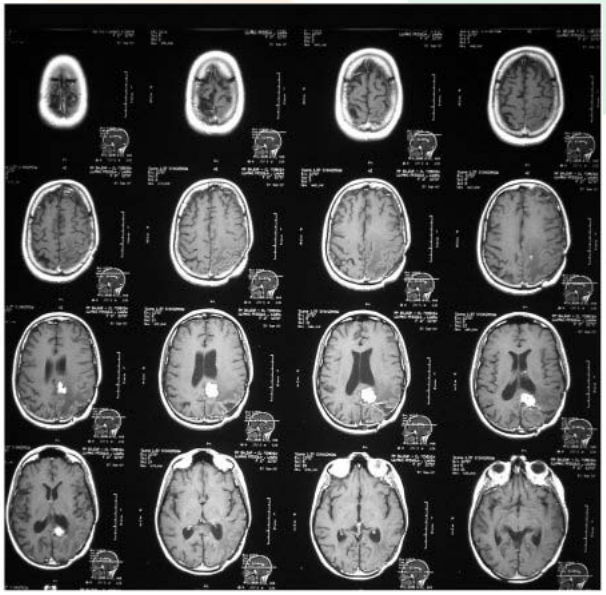
Immunocytochemical analysis of brains from nude mice inoculated with human glioma cells and treated (p.o.) with vehicle (control), Minerval® or temozolomide (TMZ)

Minerval® in humans



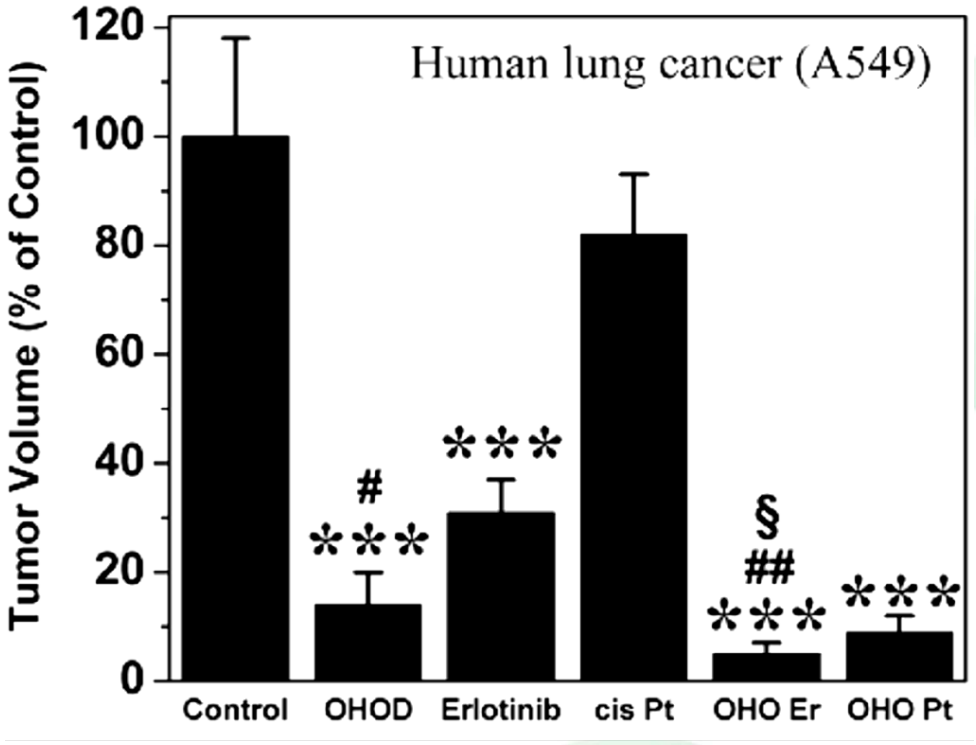


Before Treatment

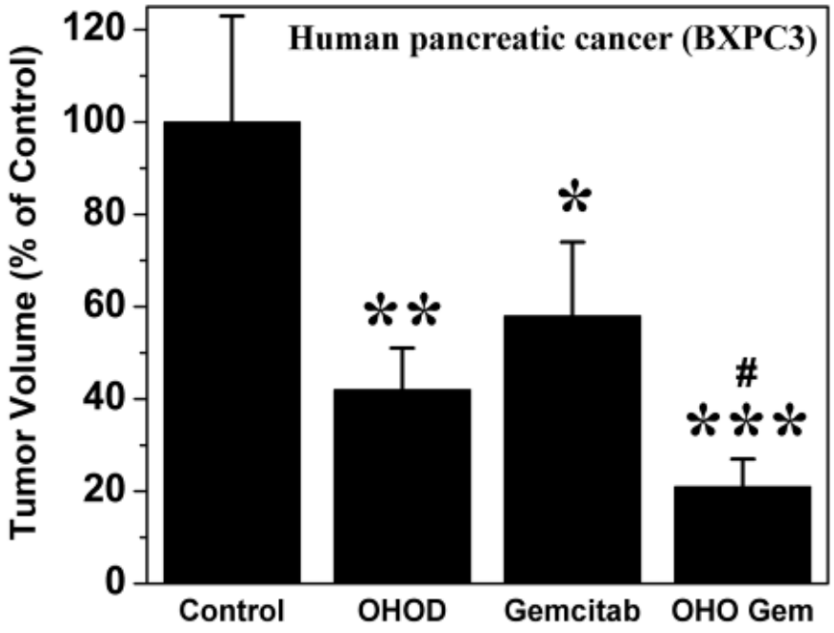


After Treatment

Effect of Minerval® (OHO/D), Erlotinib & Cis Platinium (cis Pt) in LUNG tumours (A549)

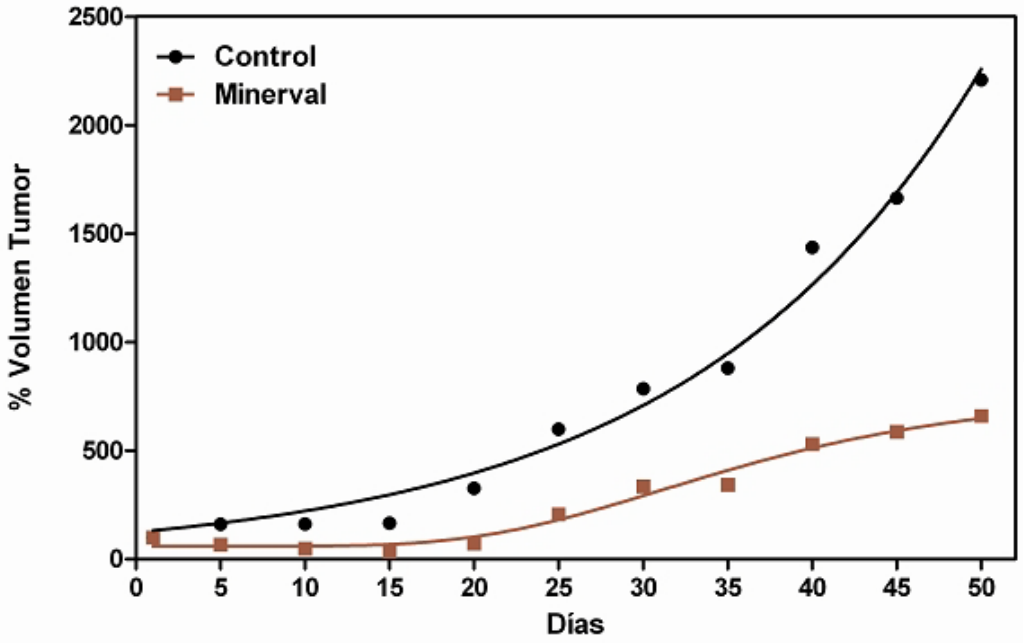


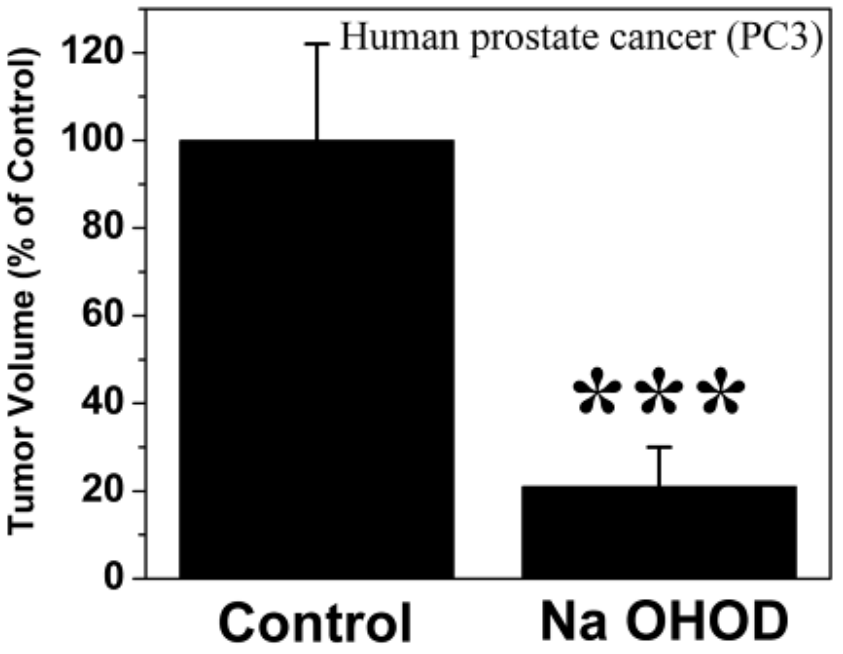
EFFECT OF MINERVAL® ON HUMAN PANCREATIC CANCER CELLS



Effect of Minerval® (OHO/D) & Gemcitabine (Gem) in PANCREAS tumours (BXPC3)

EFFECT OF MINERVAL® ON HUMAN PROSTATE CELLS





Minerval® in Cancer: novel MOA

Regulation of Membrane Lipids structure & composition (1)

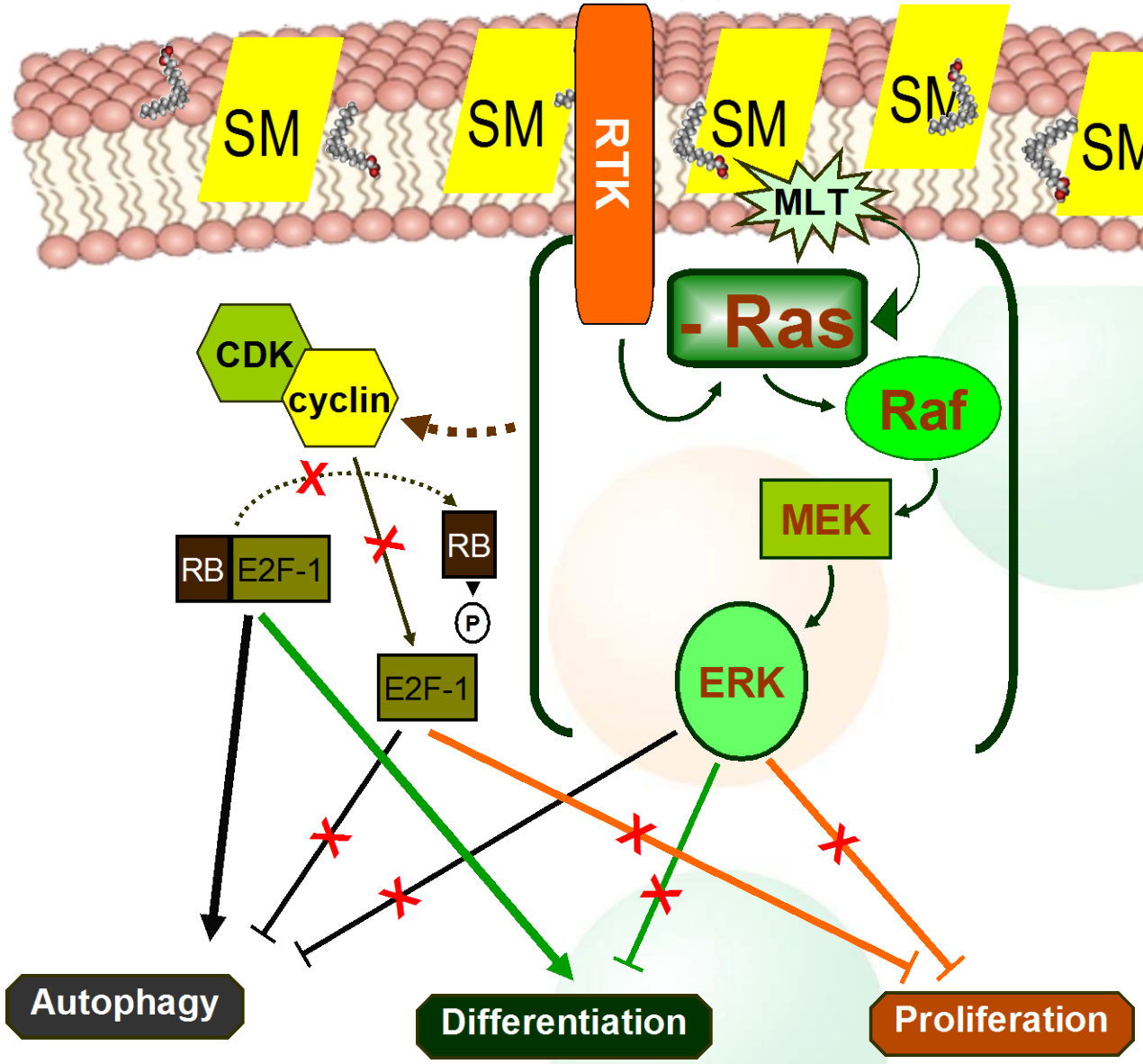
Ras / MAP kinase pathway inhibition (2)

Cell cycle arrest (3)
(DNA synthesis inhibition)

cell differentiation (4)

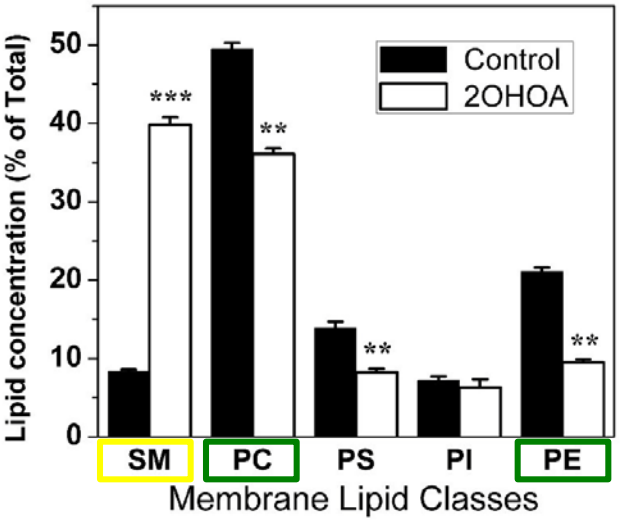
p27/RB associated Autophagy (5)

Minerval®: Mechanism of Action in Glioma

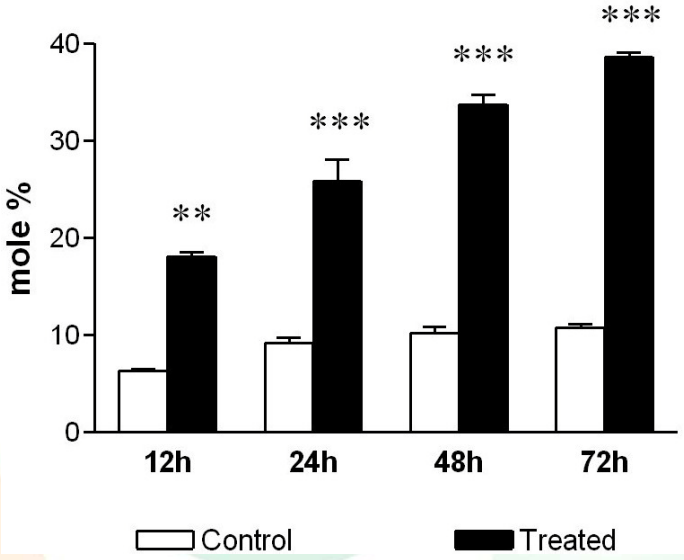


(1) Minerval® regulates membrane-lipid structure & composition in glioma cells

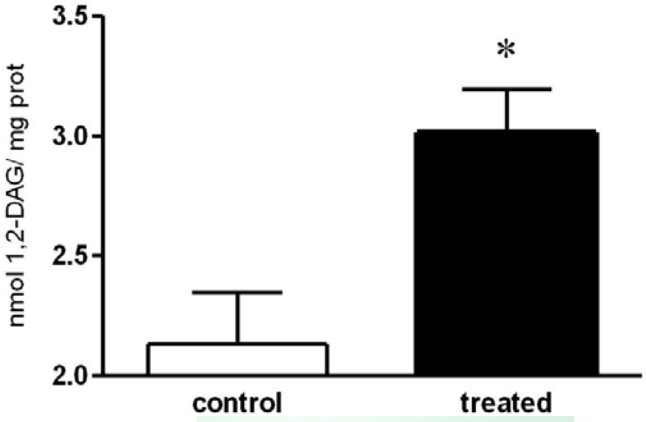
Minerval® on membrane lipids in human glioma cells (U118)



Levels of SM, phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) after 72-h treatments with Minerval® (2OHOA)

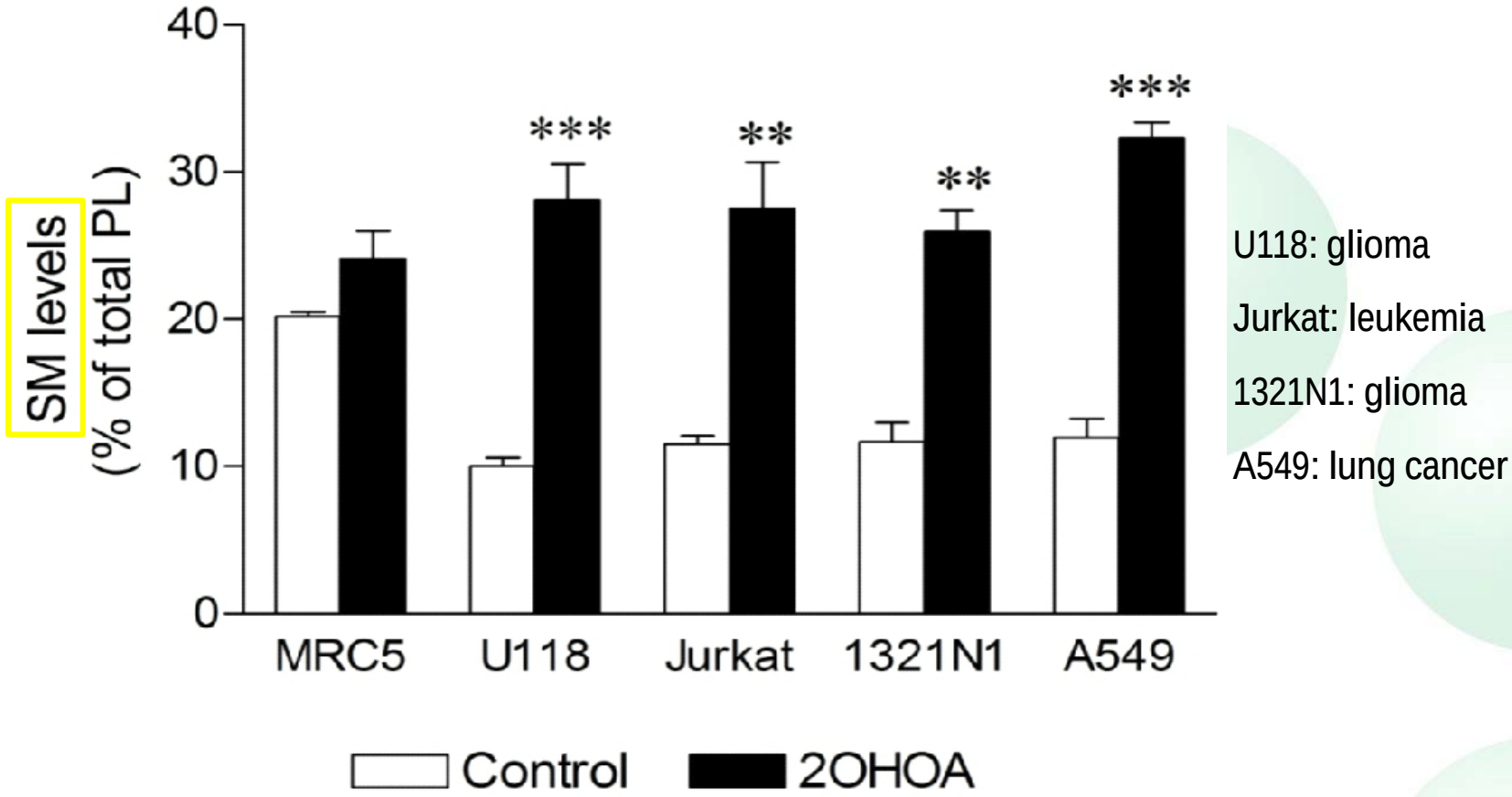


Time-dependent changes in sphingomyelin (SM) content in U118 Cells after treatment with Minerval®



Increase induced on diacylglycerol (DAG) levels after Minerval® treatments for 72 hours

Effect of Minerval® on SM levels in normal & cancer cells

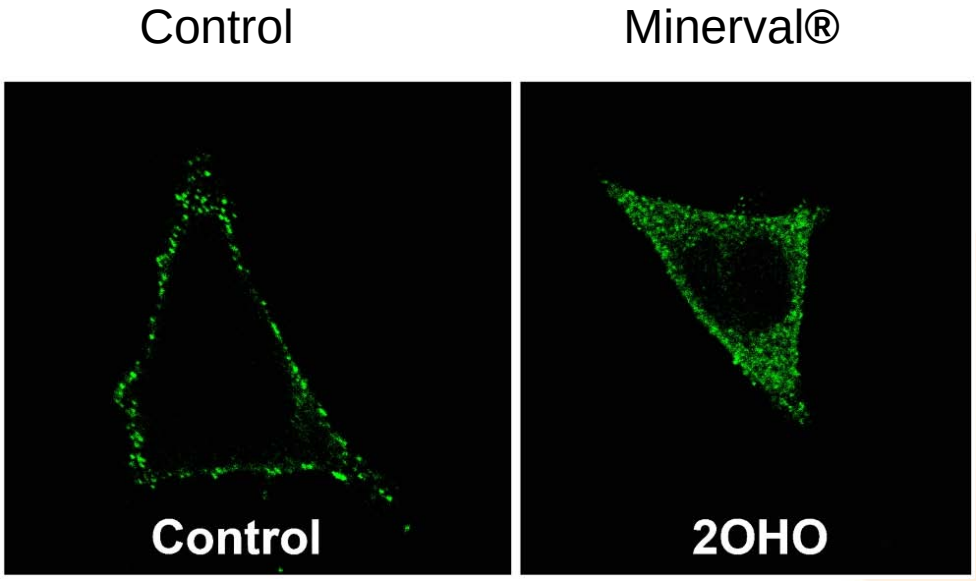


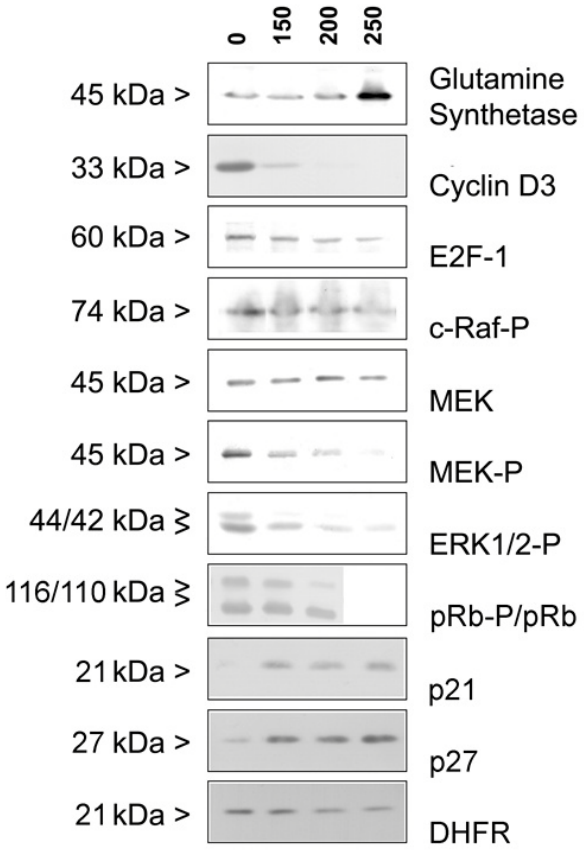
1) All Cancer cell lines studied show a marked reduction of SM levels compared to normal (MRC5) cells (open bars)

2) Minerval® induces a very important rise in the levels of SM in all cancer cells studied, returning SM levels in membranes to "normal" values (solid bars), while changes in SM are not significantly different in normal cells (MRC5)

(2) Minerval® induces translocation of Ras from membrane to cytosol, inactivating the Ras/MAP Kinase signaling pathway

Minerval® induces membrane-to-cytosol translocation of Ras in GLIOMA cells (SF767) ...

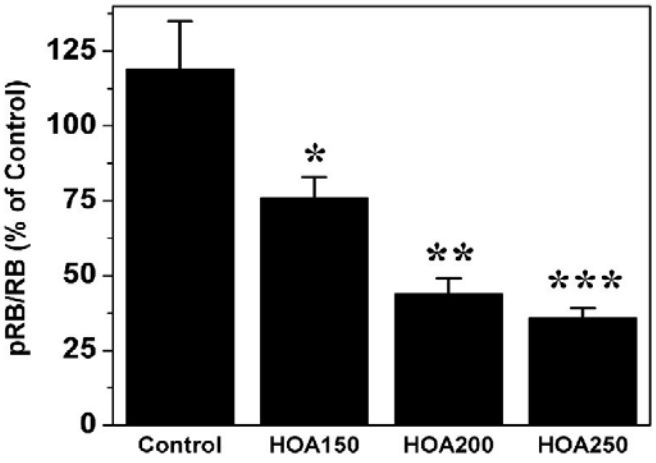


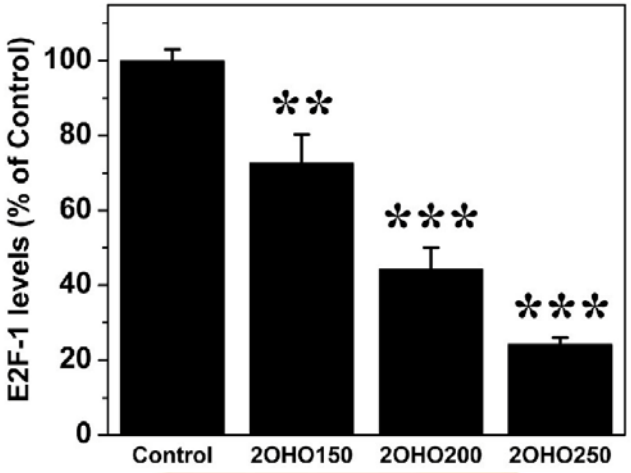


... inactivating the Ras/MAPK pathway, which was demonstrated measuring the levels of Raf (MAPKKK), MEK (MAPKK), MAPK (ERK1/2) and their corresponding phosphorylated forms. In this context, 2OHOA did not inhibit the expression of these proteins, but it induced marked and significant reductions of their phosphorylation, which is associated with their low activity state.

(3) Minerval® reduces cell proliferation by down-regulating E2F-1 and DHFR (necessary for DNA synthesis), due to pRB hypophosphorylation

Minerval® inhibits pRB/RB, E2F-1 & DHFR expression (SF767)

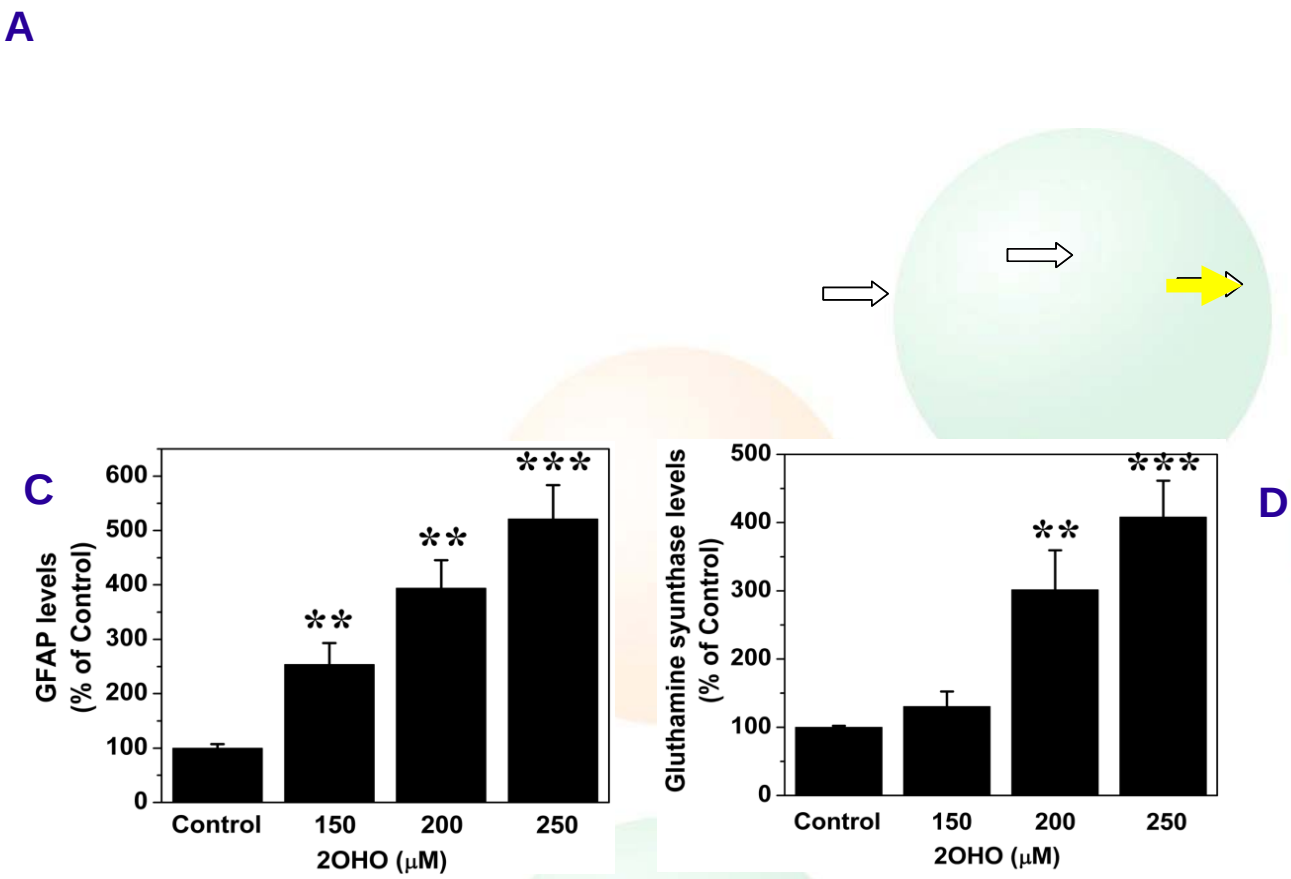




Changes produced by Minerval® (HOA, 2OHO) on cell membrane composition alter the Ras/MAP kinase pathway, which in turn regulates the Cyclin-CDK/RB-E2F-1 pathway. This leads to a reduction of pRB phosphorylation and to an important knockdown of E2F-1, which induces its own expression and those of Cyclins, replication factors, and growth factor receptors, including an important downregulation of DHFR (necessary for DNA synthesis). The final result is inhibition of glioma cell proliferation and later induction of differentiation and autophagy.

(4) Minerval® induces cell differentiation

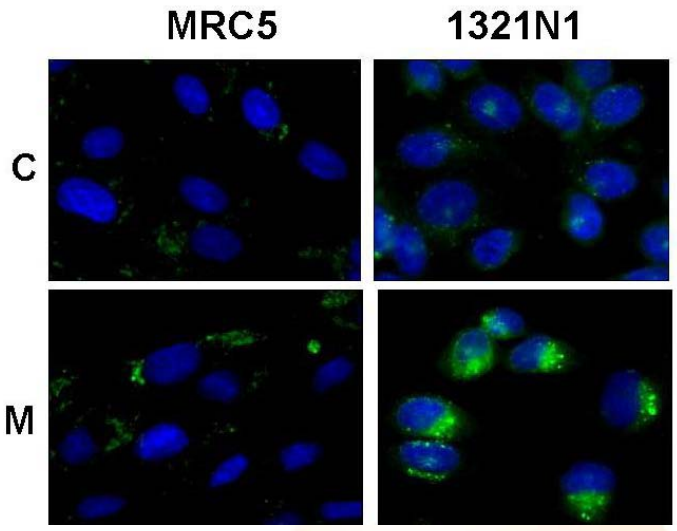
Minerval® induces glioma cell differentiation



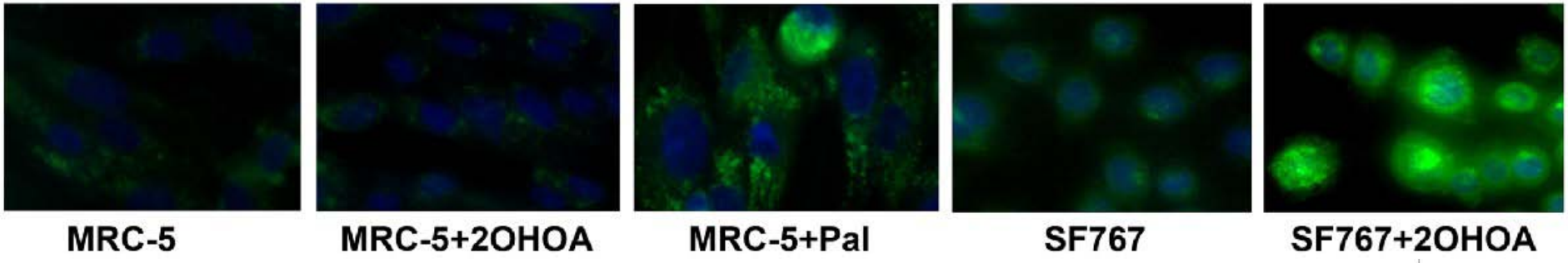
Minerval® induces glioma cell differentiation, as shown by the morphological and molecular changes induced by this drug. A, Optical microscopy in various glioma cell lines. B, Expression of the differentiation marker glutamine synthase (yellow arrow) in tumors from mice bearing human gliomas and treated with Minerval® (2OHOA; *in vivo*); C and D show the overexpression of the differentiation markers GFAP and glutamine synthase in human glioma (SF767) cells, typical of mature glial cells, which are lost during the malignant transformation into glioma cells.

(5) Minerval® induces selective cancer cell death by autophagy

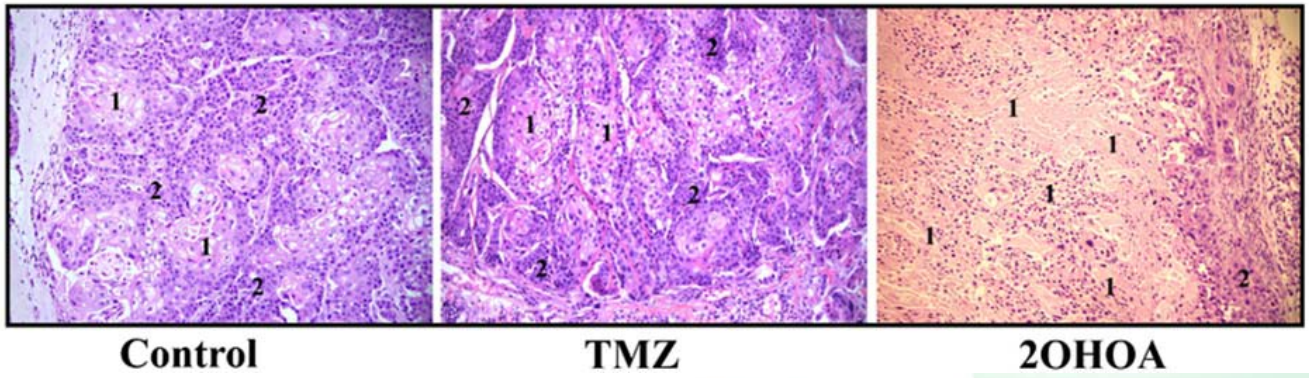
Minerval® induces AUTOPHAGY in Glioma cells but not in normal cells

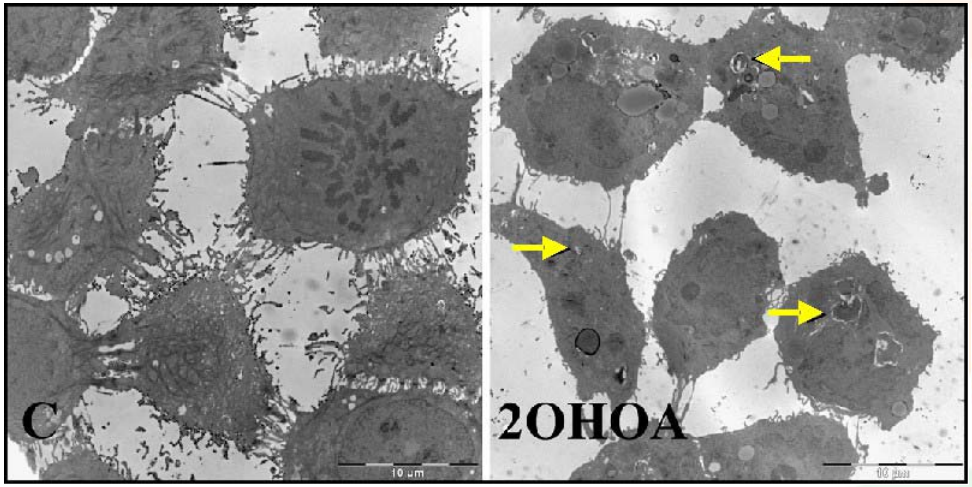


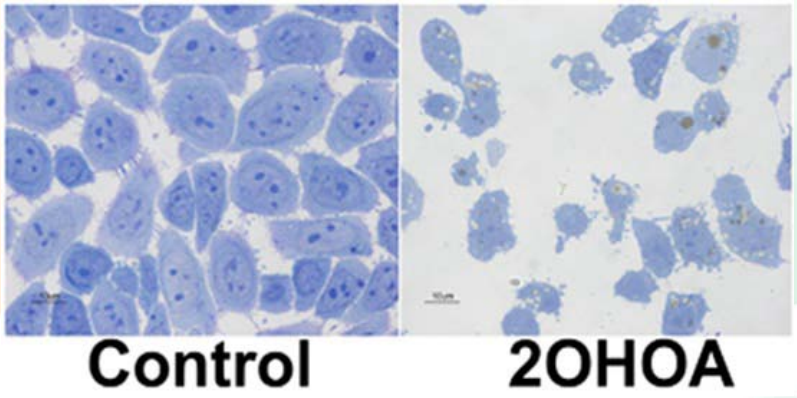
2OHOA selectively induced autophagy in human glioma 1321N1 cells (top) and SF767 cells (bottom) but not normal (MRC-5) cells. Fluorescence of lysosome/autophagosome labelled with Lysosensor in MRC-5 cells in the presence or absence (bottom left) of 2OHOA or palmitic acid (Pal), a known inducer of ER stress and autophagy. The last 2 panels (bottom right) show the effect of 2OHOA in glioma cells



Minerval® induces AUTOPHAGY in Glioma cells







Top: ex-vivo tumor samples, where 1=live cells & 2=dead cells

Bottom left: autophagosomes. Transmission electron micrographs of human glioma (SF767) cells incubated in the presence or absence of 2OHOA. The arrows in treated cells show some of these autophagosomes.

Bottom right: cell fragmentation observed in glioma cells after treatment with Minerval®

MINERVAL®

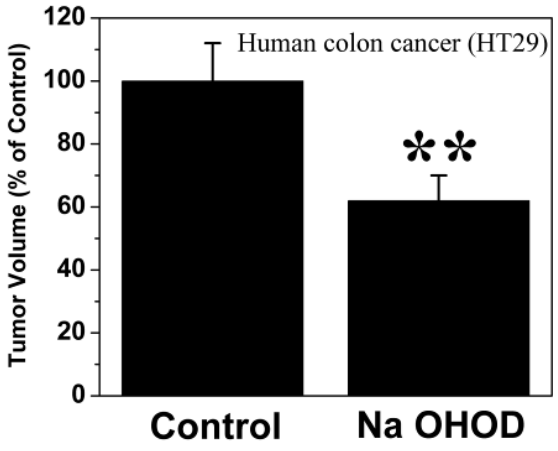
High Efficacy

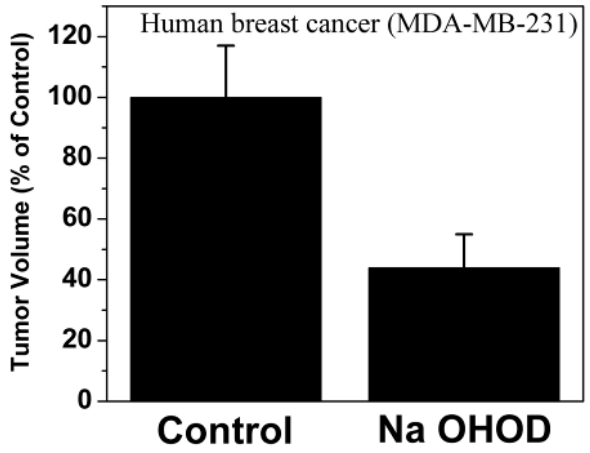
+

Absence of Toxicity

+

Oral Administration



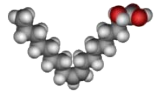


Minimum Lethal Dose not determined!
> 3.000 Mg/Kg

Very promising profile
in Cancer treatment !

Strong global IP position

MINERVAL®



Invention protected by 2 global Patent Families (2002 & 2009) covering the cancer applications of the 2OHOA and structural analogue molecules

Patent granted in Europe, USA, Japan, China, Russia & Mexico.

Patent applications under evaluation in Canada, Brazil,

Minerval® today:

- ✓ **Preclinical** program completed
- ✓ Ready to move into **Clinical Trials** (H2 2011)
- ✓ Filed for **Orphan Drug** status in EU and USA (glioma)
- ✓ Looking for collaborations with **leader global oncology players** for completion of **clinical development** in **oncology**

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