Programa Cooperación Farma-Biotech

8º encuentro (7 de mayo de 2013)

Minerval for the treatment of glioma and other solid tumors

LIPOPHARMA

Lipid Science for life

Vicenç Tur. CEO, Co-founder

Madrid, 7 de mayo de 2013

GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

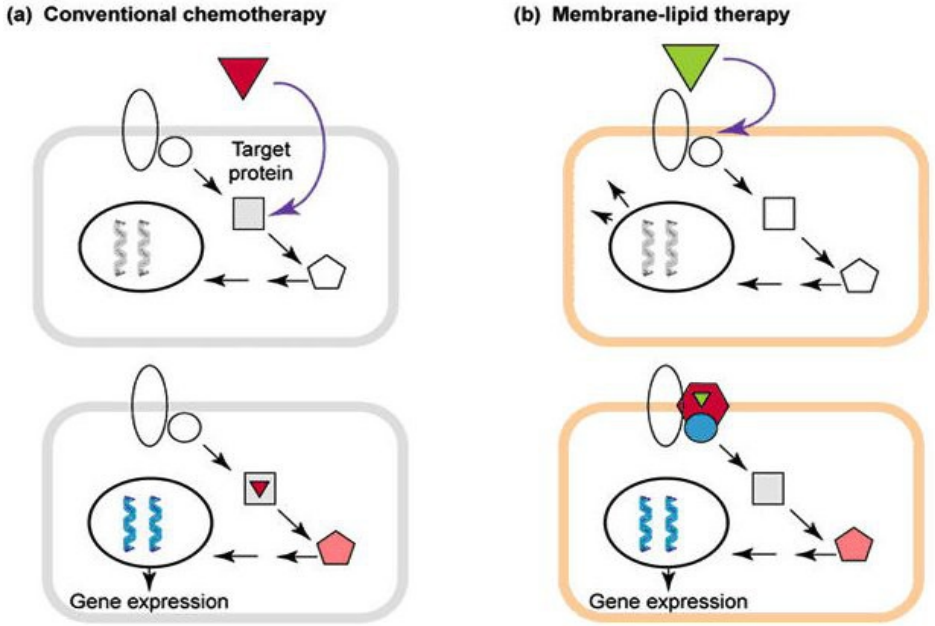
farmaindustria

Content

1. **The Company**
2. **The Technology platform: TLM**
3. **The Product**
   a) Target Indications
   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. **Partnering Opportunities**

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

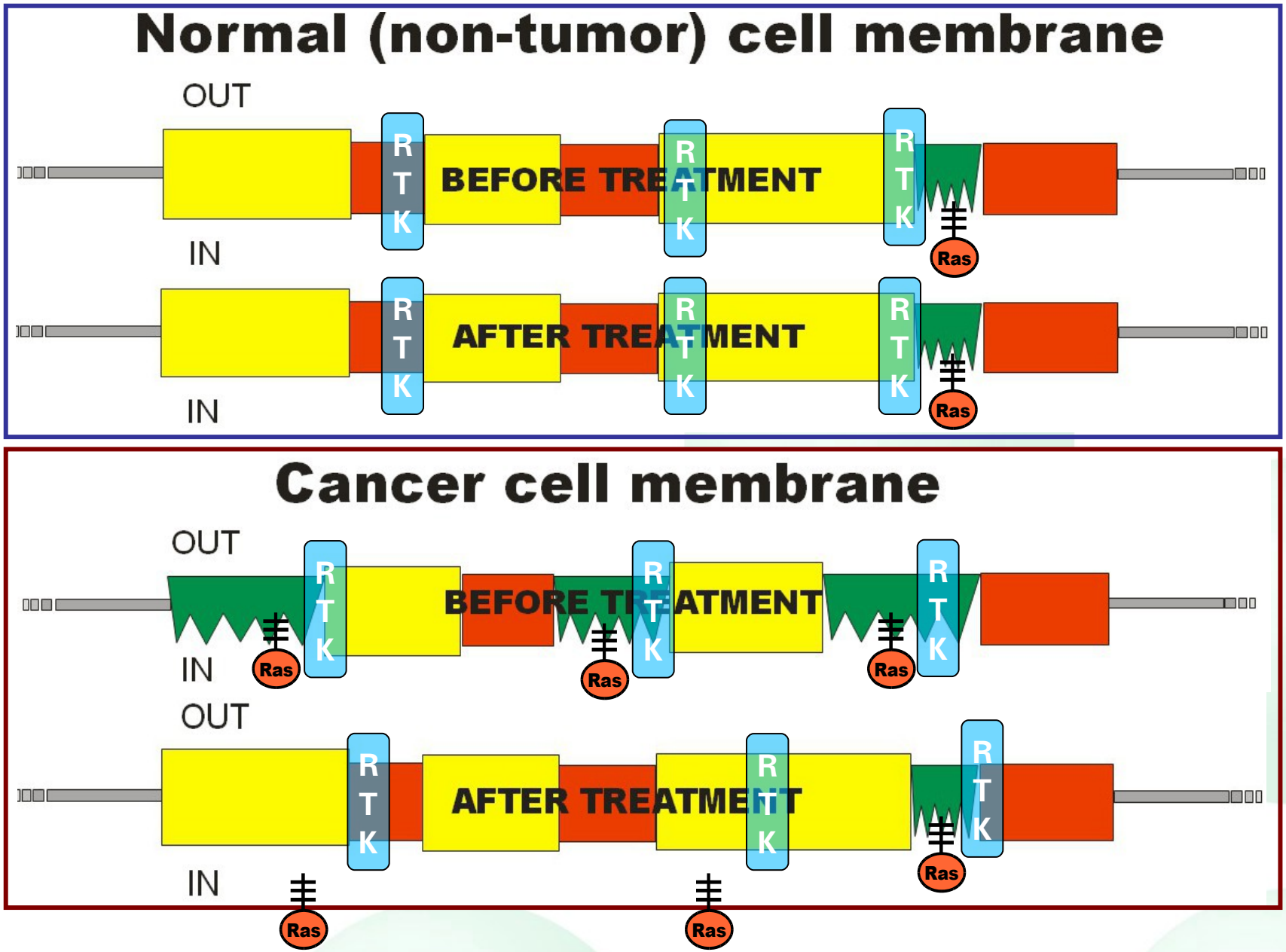
Membrane Lipid Therapy (MLT)



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

MLT is a disrupting innovative therapeutic approach consisting in the design of molecules that target membrane lipids instead of proteins (intracellular, receptors, growth factors,…) The changes in the lipid composition of membranes alters the structures they form and determine the localization and activity of membrane proteins and the messages they propagate. These changes are highly specific and are based on structure–function principles

Minerval: MLT in Action



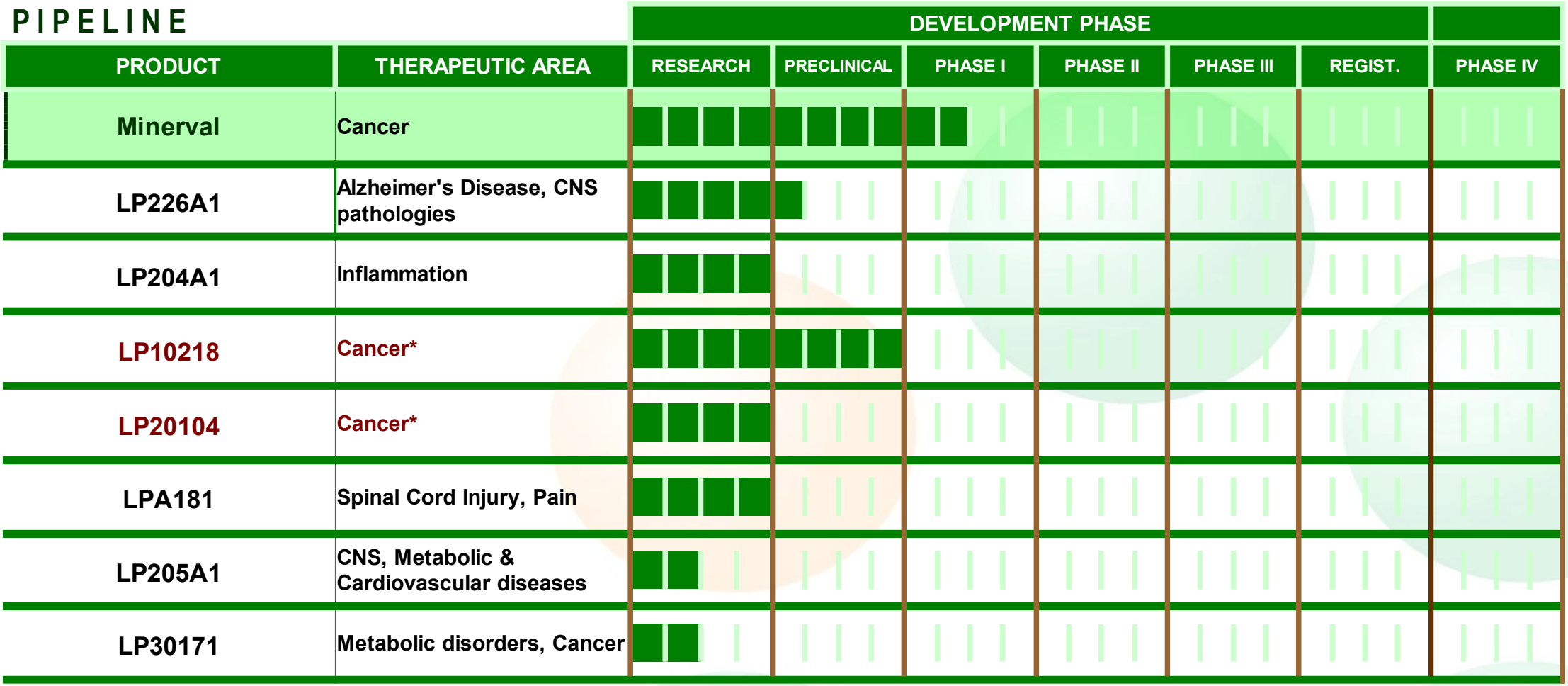
High SM (raft) membrane domains

High DAG membrane domains

High PE & PC membrane domains

MLT drugs are designed to influence and **regulate lipid organization in cell membranes** based on **structure–function principles**, inducing a concomitant modulation of **membrane protein localization and activity**, which finally induces changes in cell signaling and gene expression.

Therapeutic products in development based on the MLT



* partnered with Ability Pharmaceuticals

Minerval for the treatment of glioma…

1. Based on an **innovative Technology/Scientific platform**:

   Membrane Lipid Therapy (MLT)

2. **Novel MOA**: **regulation of membrane lipids** → **Multi-pathway modulation**

3. High **specificity**; very high **efficacy** in glioma (in cells & animals). **Non Toxic.**

4. **ORAL formulation.** Crosses the **BBB**

5. Simple Chemistry. **Stable** at **ambient** for +24 months

Minerval, a new class of lipid regulator, multi-pathway anticancer drug

for tumors with **sphingomyelin metabolism alterations**

Specific activator of sphingomyelin synthase (SMS) [1]

Selective** regulation of **lipid composition** in cancer cell **membranes:

▲SM; ▲DAG; ▼PE; ▼PC [2]

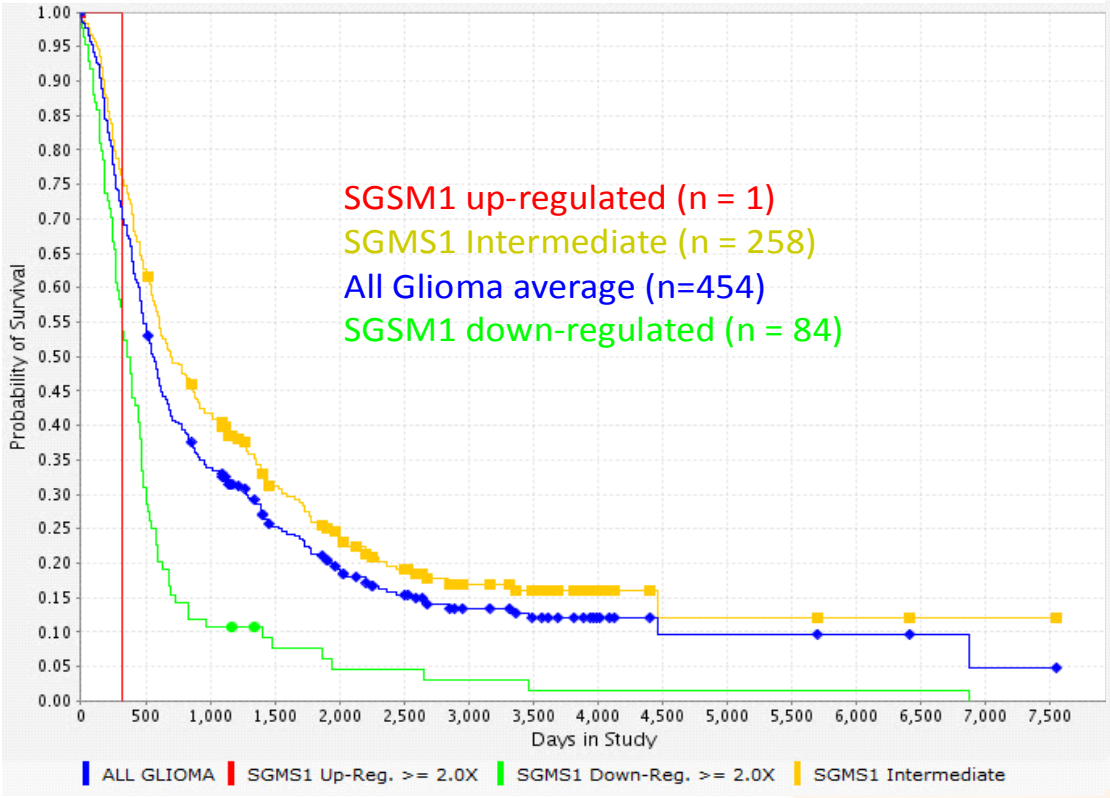
PKC/Cyclin CDK
Regulation [4]

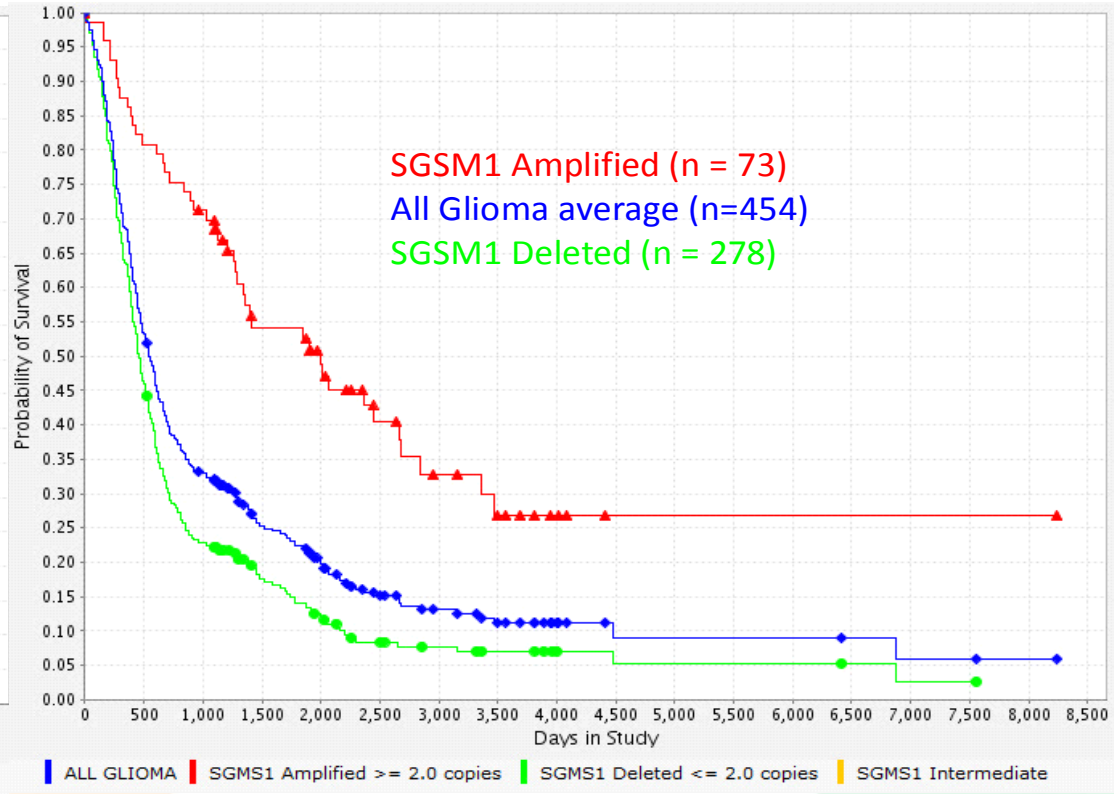
Ras traslocation
↓
MAPK – PI3K/Akt
pathway normalization [3]

Cell cycle arrest [▼DHFR]
& **differentiation** [4]

selective **Autophagy** [5]

Effect of SMS1 genetic alterations on glioma patients' survival
(Differential SGMS1 Gene Expression & Copy Number Analysis for SGMS1)

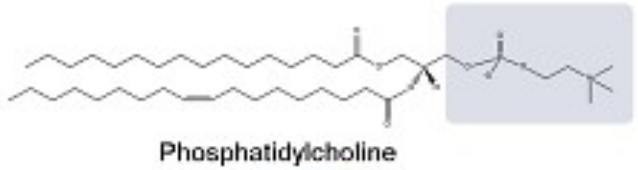


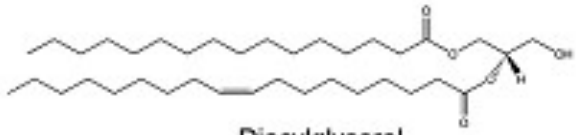


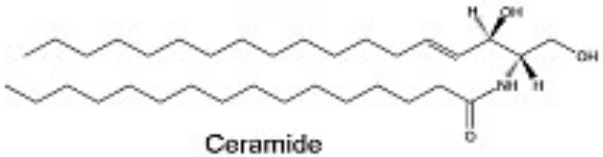
Kaplan-Meier survival plot for patients with glioma (n=454) *vs* sphingomyelin synthase 1 (SGMS1) expression (**left**) or gene copy number (**right**). Human glioma cells have low sphingomyelin (SM) levels (Barcelo-Coblijn et al., PNAS 2011; 108:19569-19574) and Minerval-induced normalization of SM levels causes glioma cell death. The left panel shows that down-regulation of SGMS1 is associated with a marked and significant ($P = 2 \times 10^{-10}$) reduction in the life-span of glioma patients. The right panel shows that deletion of the SGMS1 gene occurs in about 61% of all glioma patients and is associated with a significant reduction in their life-span, whereas patients with more than 2 copies of the SGMS1 gene have an increased life span and ca. 25% probability to live over 20 years.

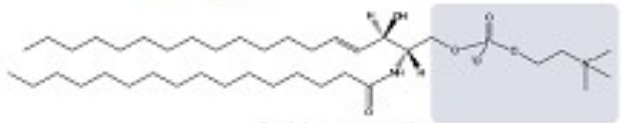
National Cancer Institute. 2005. REMBRANDT <http://rembrandt.nci.nih.gov>. October 2012

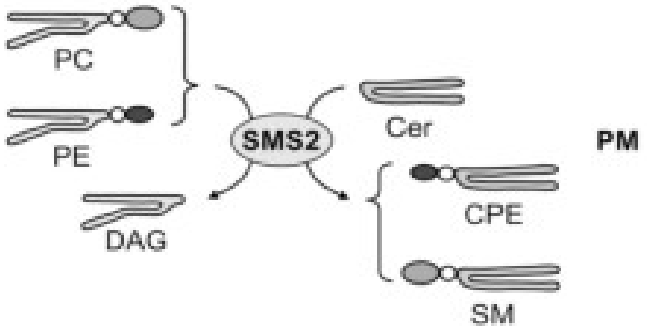
Sphingomyelin synthase (SMS) catalyzes SM synthesis

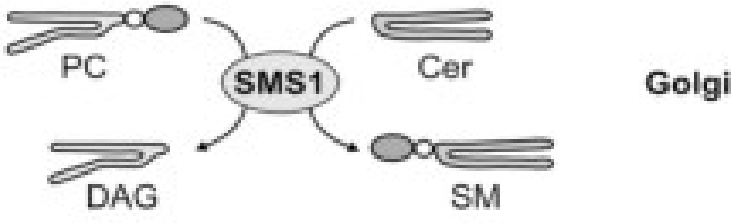






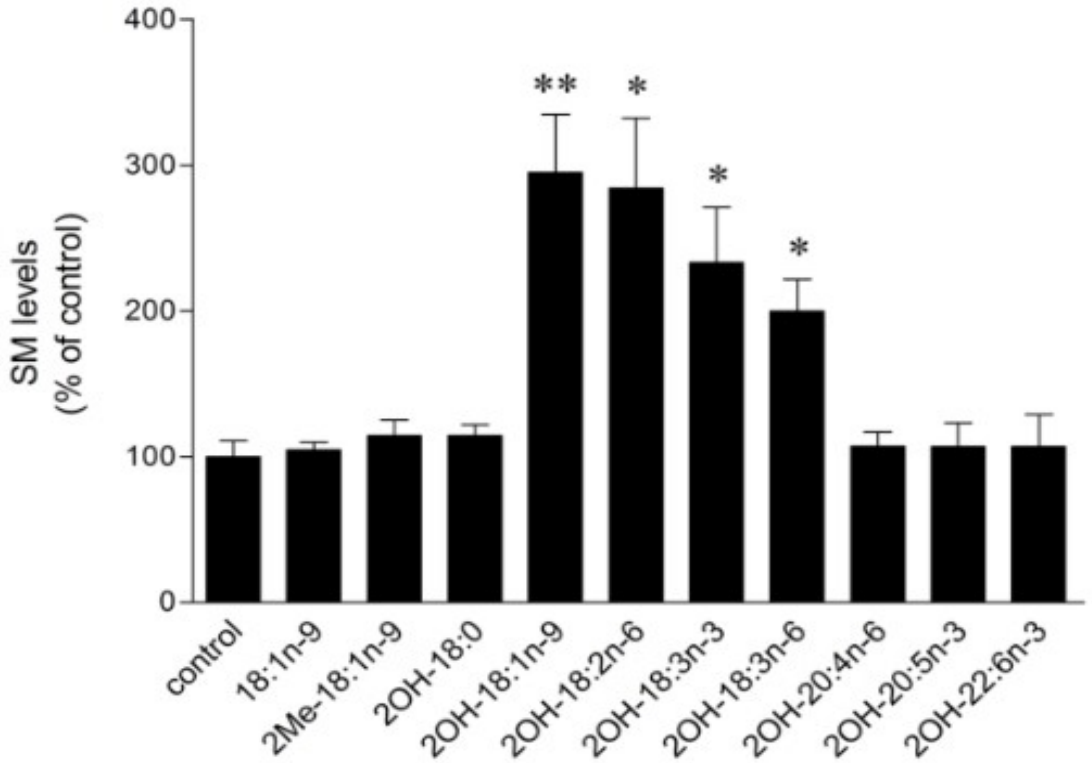






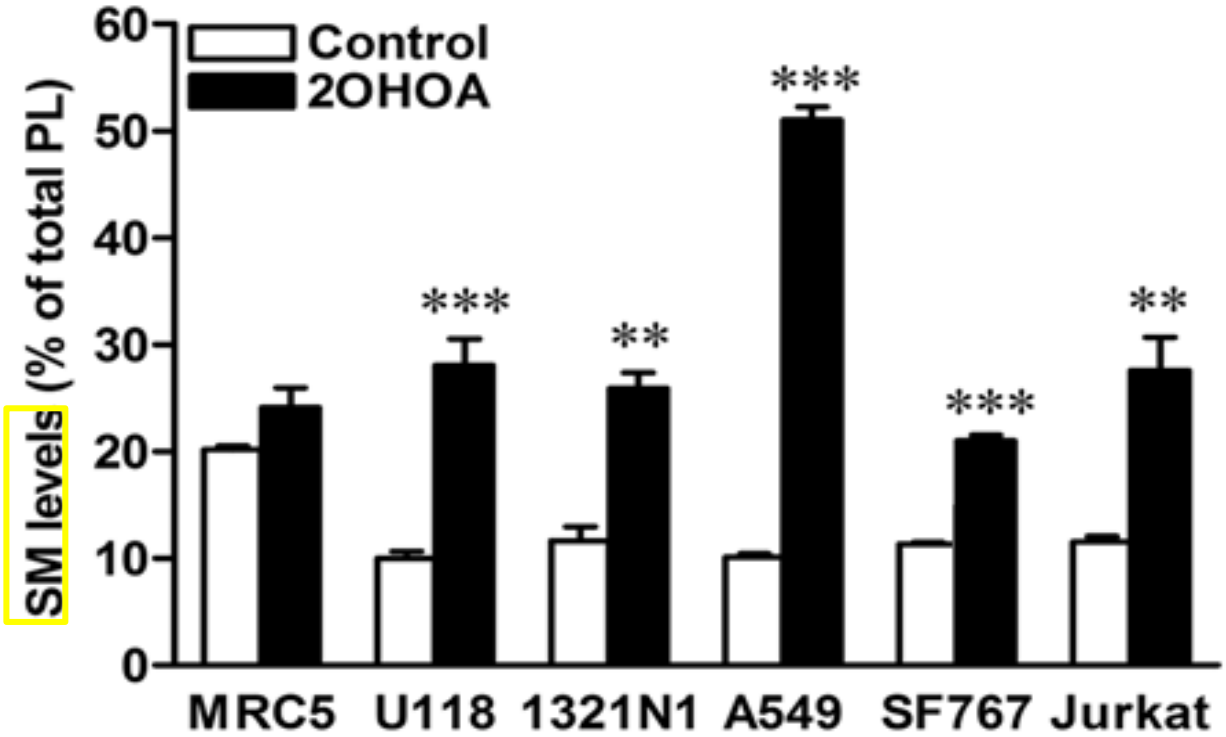
SMS mediates the synthesis of sphingomyelin (SM) and DAG using ceramide and phosphatidylcholine (PC) or phosphatidyl-ethanolamine (PE) as substrates. Because this enzyme can work forth and back, in cells where the levels of substrate (e.g., PE) are high and product (e.g., SM) low, 2OHOA activates SMS (e.g., cancer cells). By contrast, when membrane levels of PE are low and SM are high, SMS cannot be activated by 2OHOA (e.g., normal cells and tissues).

Minerval Structure-Activity Relationship in SMS activation



Structure-function relationship in **SMS activation**. Only fatty acids with 18 carbon atoms, at least one double bond and a hydroxyl (but not other) moiety on C-2, induce SMS activity in human glioma (U118) cells after 24-h incubations with 200 microM of the indicated compound (*Barceló-Cloblijn et al., PNAS, 2011*)

Effect of Minerval on SM levels in normal & cancer cells



MRC5: non-tumor

U118: glioma

1321N1: glioma

A549: lung cancer

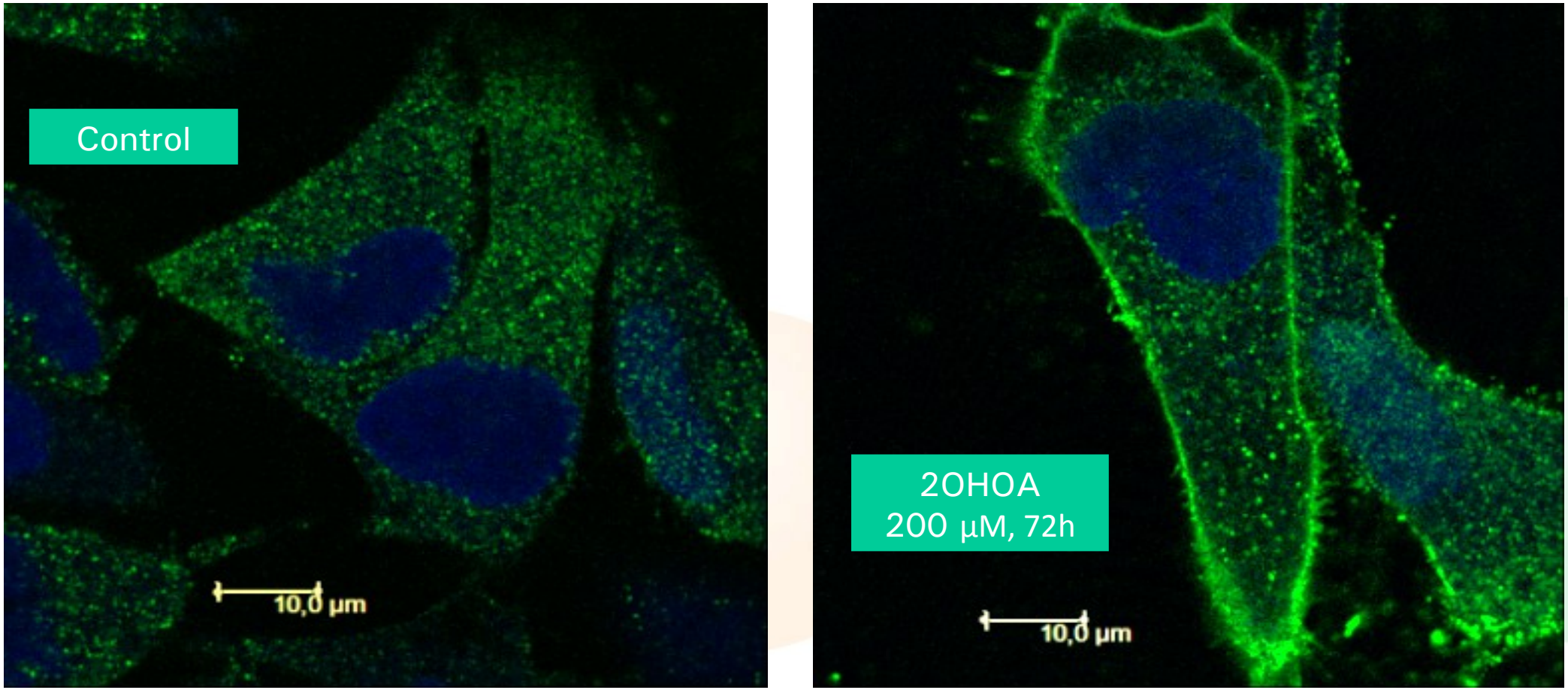
SF767: glioma

Jurkat: leukemia

1) All 5 cancer cell lines studied show a marked reduction of SM levels compared to normal (MRC5) cells (open bars)

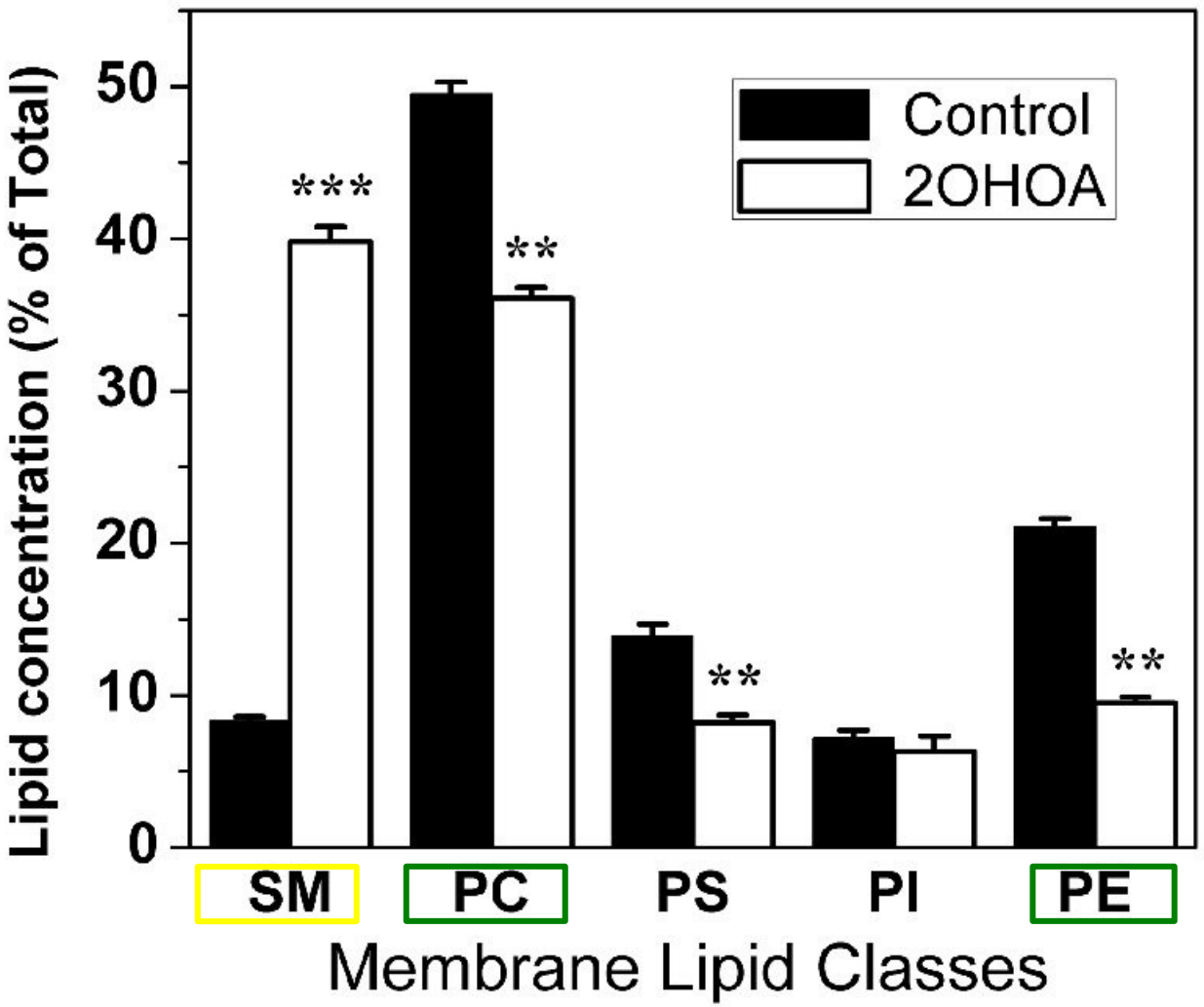
2) Minerval induces a very important rise in the levels of SM in all cancer cells studied, returning SM levels in membranes to "normal" values (solid bars). Changes in SM content in normal cells (MRC5) are not relevant

SM increases mainly at the plasma membrane

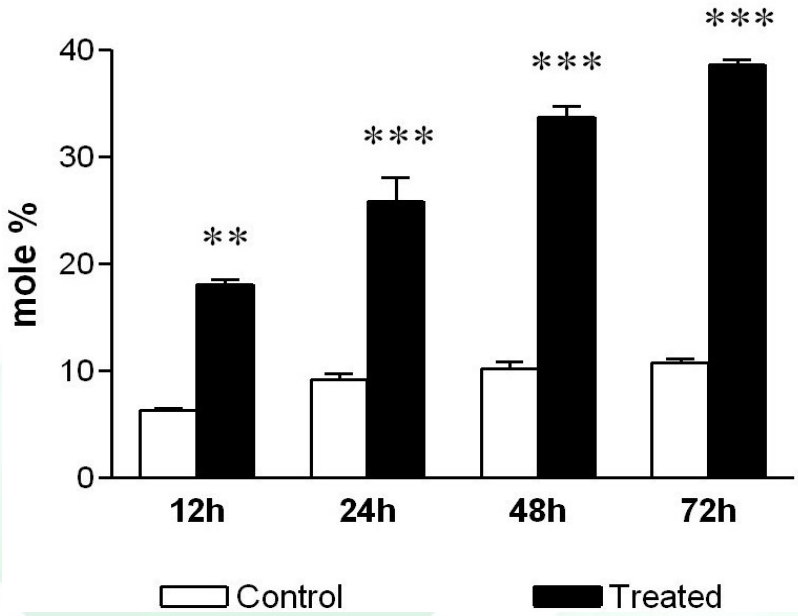


In human glioma (U118) cells, treatment with Minerval (2OHOA) induced a very important increase in the levels of membrane SM, as detected with lysenin by confocal microscopy. Bar = 10 microM.

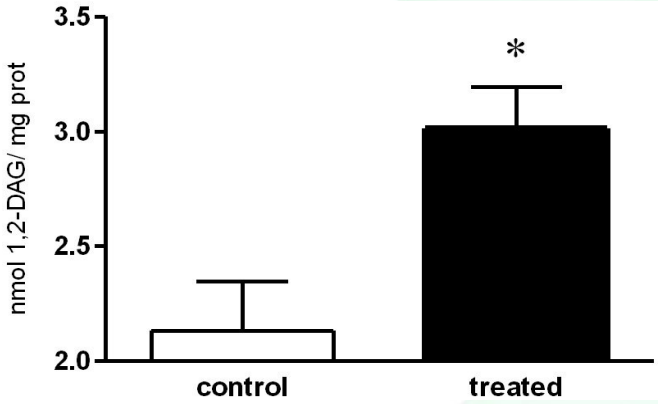
Minerval on membrane lipids in human glioma cells (U118)



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

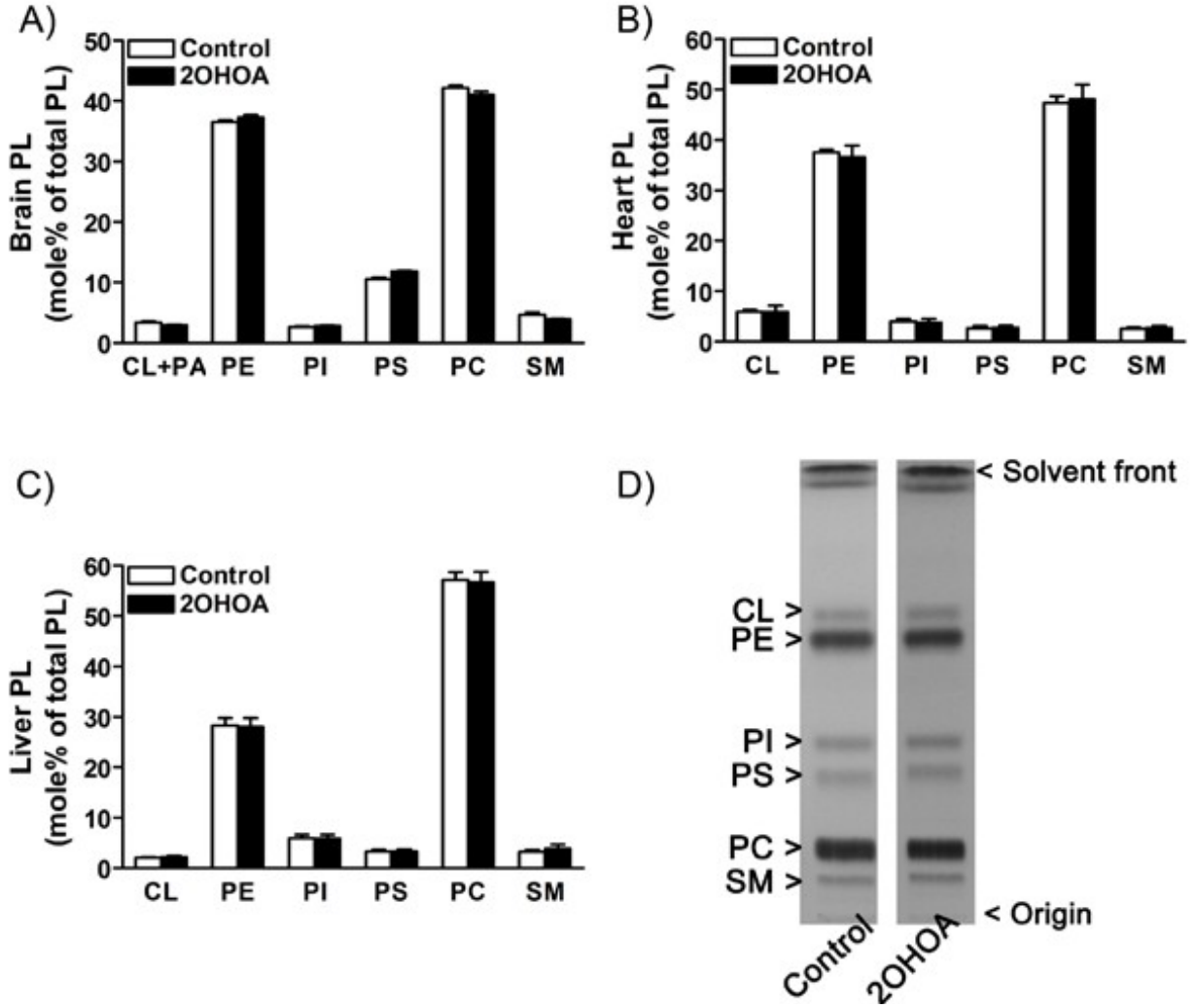


Time-dependent changes in sphingomyelin (SM) content in U118 Cells after treatment with Minerval



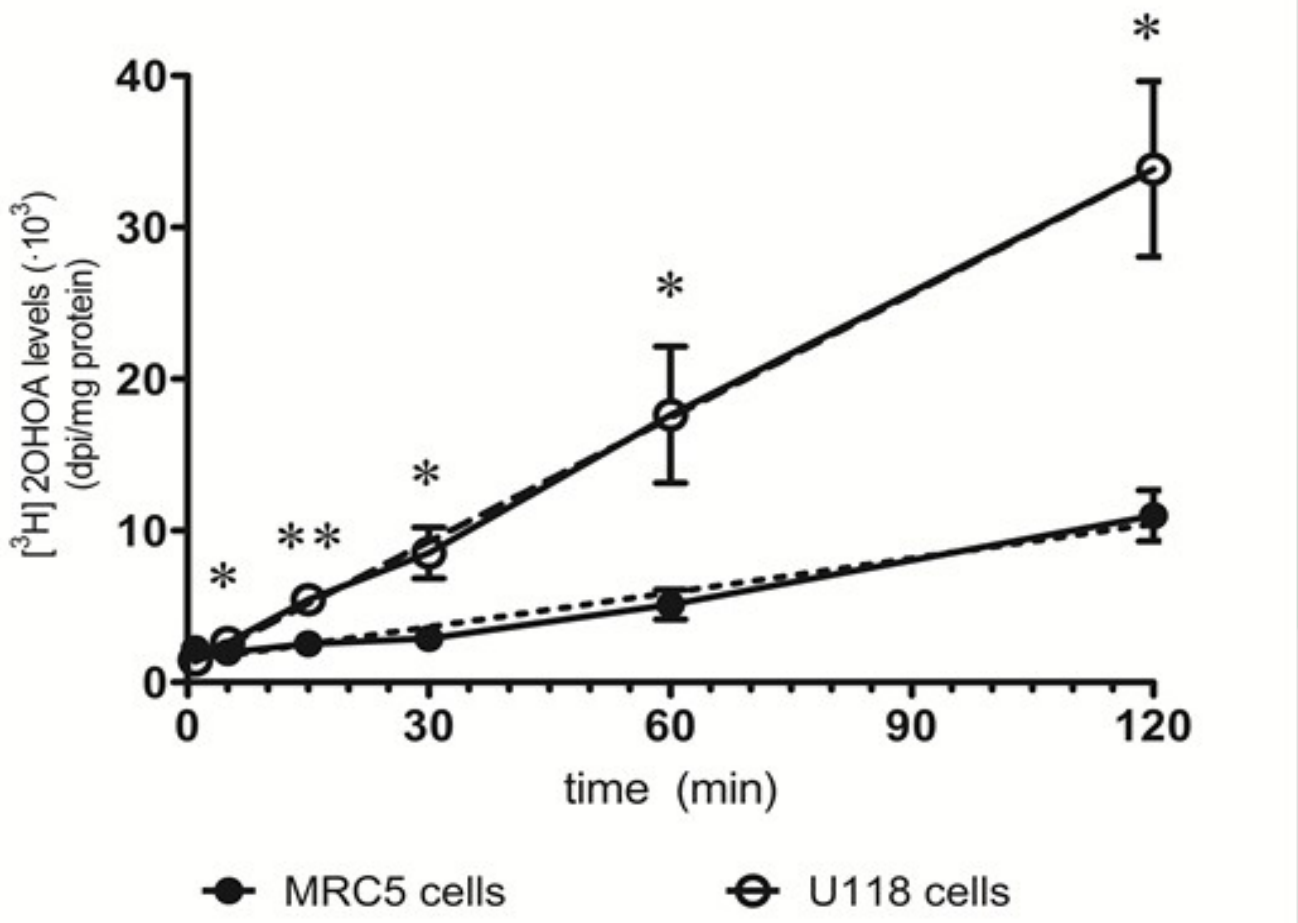
Increase induced on diacylglycerol (DAG) levels after Minerval treatments for 72 hours

No changes observed in the lipid levels of organs of mice treated with Minerval



Effect of 2OHOA treatment on brain, heart and liver phospholipid composition in mice normal tissues. (A) Brain, (B) Heart, and (C) Liver glycerophospholipid composition of mice treated with 2OHOA (600 mg kg, 10 days). Results are expressed as mean ± sem values from 5 animals. CL: cardiolipin; PA: phosphatidic acid; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; PC: phosphatidylcholine; SM: sphingomyelin

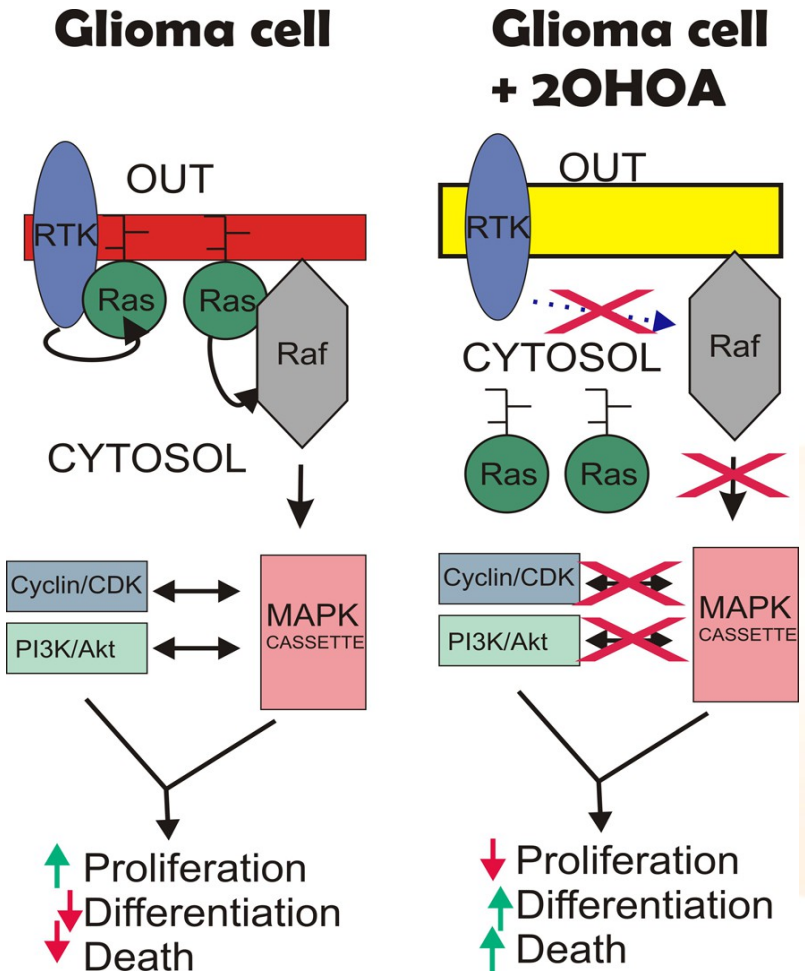
Minerval preferably incorporates into membranes of cancer cells

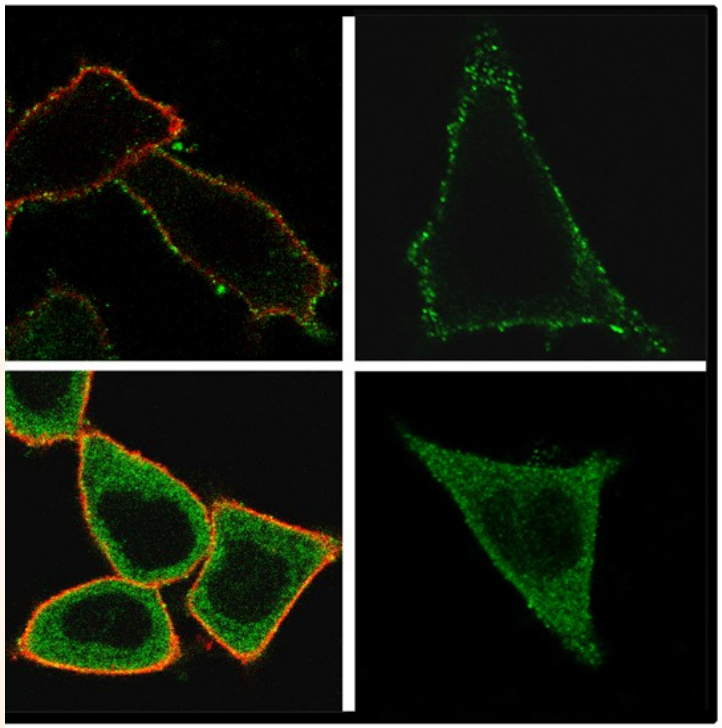


Differential incorporation of [3H]-2OHOA into U118 compared to MRC-5 cells. U118 (human glioma cells, filled circles) and MRC-5 (human lung fibroblast cells, unfilled circles) were pulse labeled for 1, 5, 15, 30, 60 and 120 min with [3H]-2OHOA (0.25 µCi/60 mm cell culture dish). *P < 0.05; **P < 0.01.

M. L. Martin *et al*. BBA Biomembranes 1828: 1405-1413 (2013)

Changes in the membrane lipid composition by 2OHOA induces membrane-to-cytosol translocation of Ras in glioma cells





Glioma cell

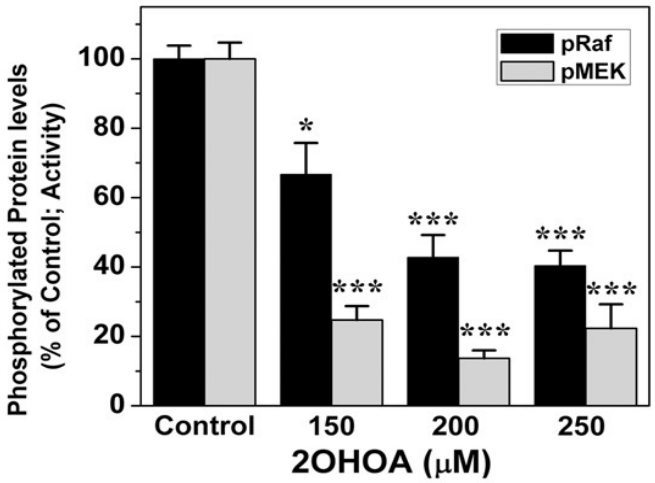
Glioma cell +2OHOA

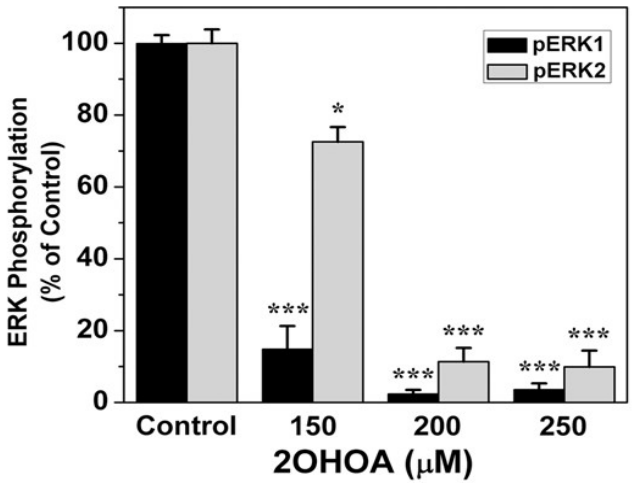
Confocal (1) Confocal (2)

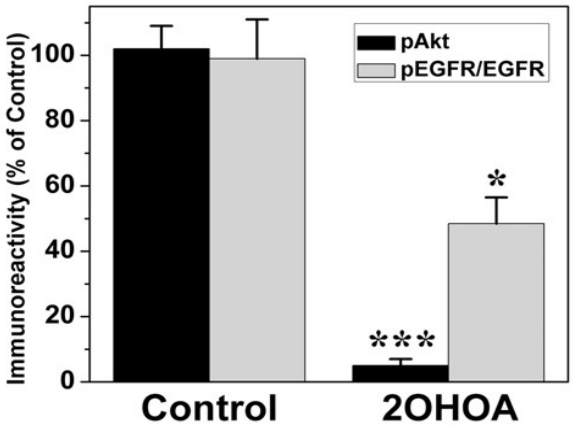
Ras

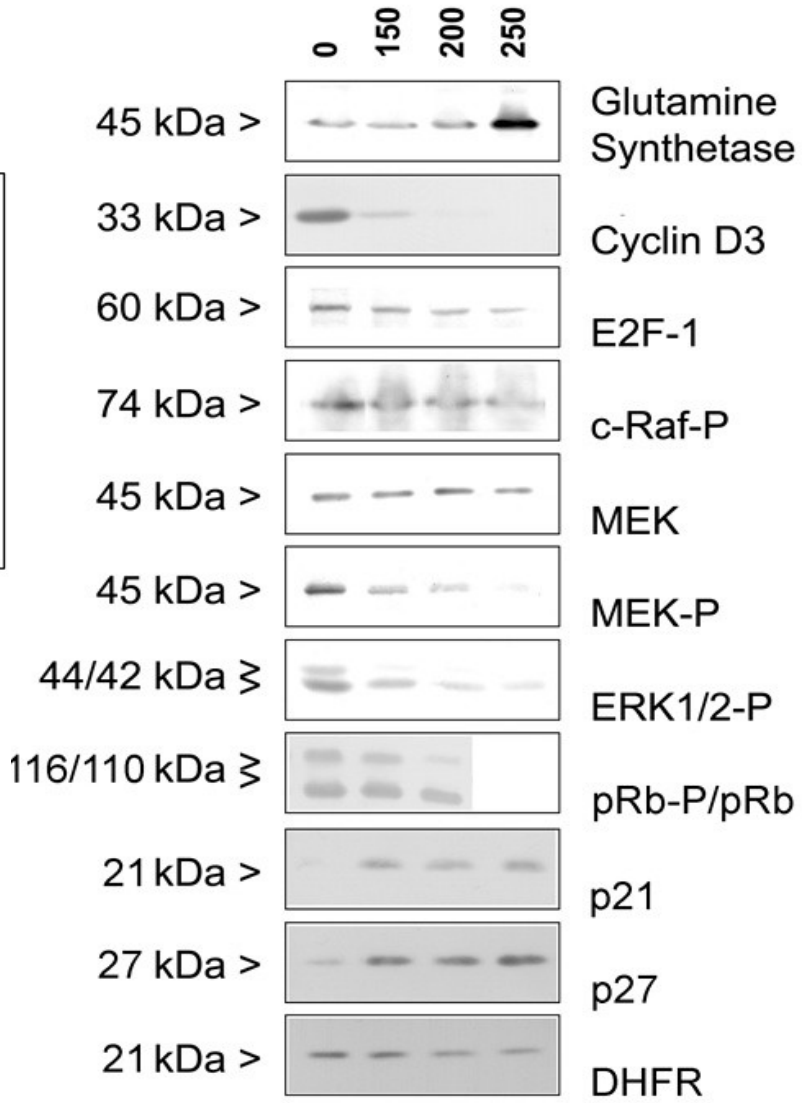
Treatments with 2OHOA change the membrane lipid composition inducing translocation of Ras from the membrane (right upper images, green) to the cytosol (lower panels). A membrane fluorescent label is shown in red. Ras translocation to the cytosol causes a shortcut of the MAP kinase pathway (left diagram).

Minerval induces inactivation of the MAPK pathway in glioma cells (SF767)



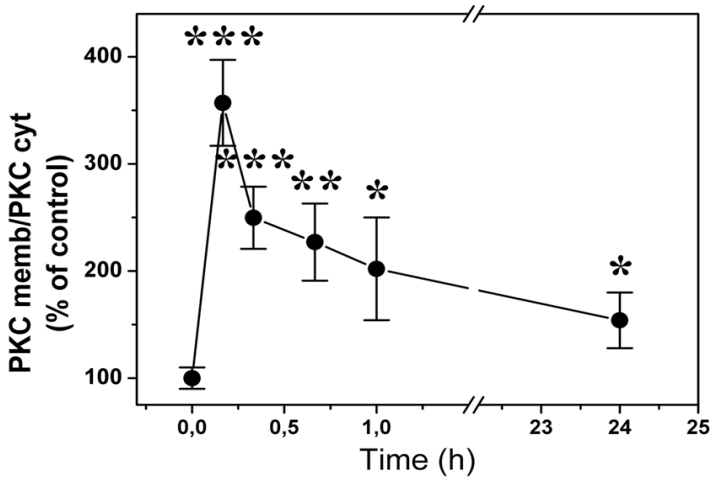


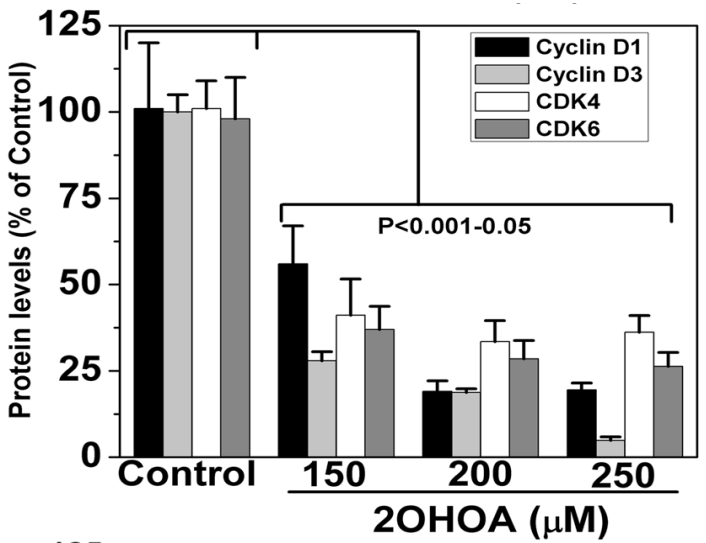


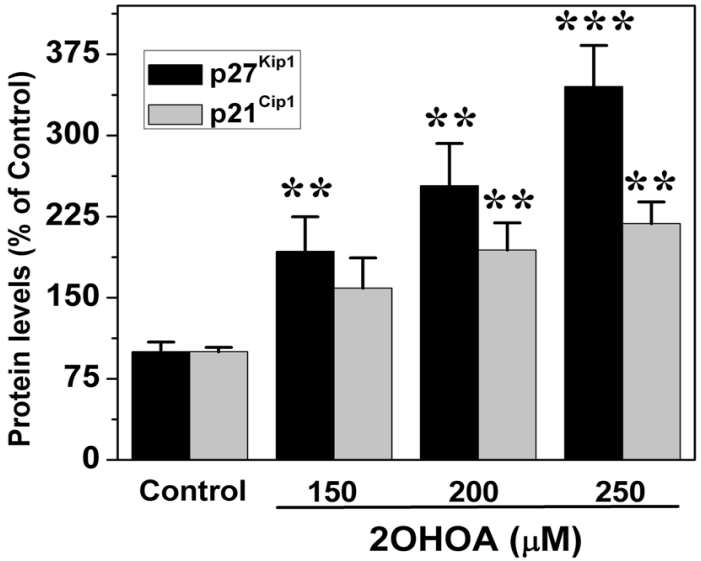


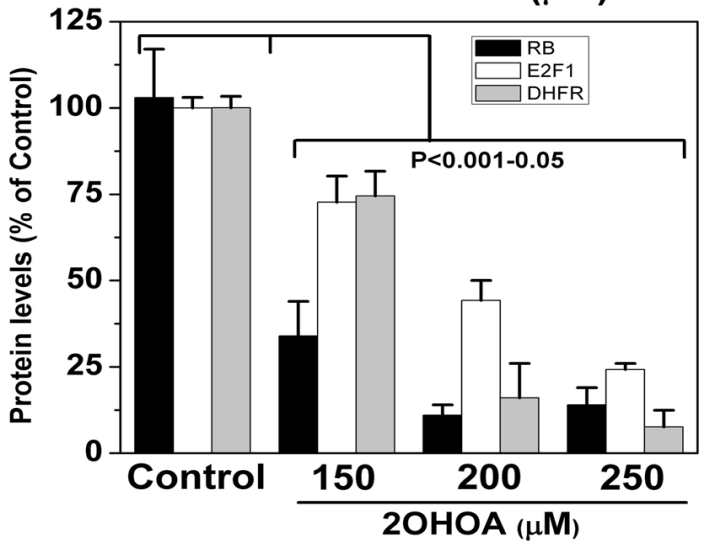
Regulation of the membrane lipid composition, responsible for translocation of Ras, induced marked and significant reductions in the **phosphorylation** status (i.e., activity) of all the proteins of the **MAPK pathway** studied. These results were also observed in tumors derived from human gliomas in immuosuppressed mice treated with Minerval (2OHOA)

Minerval inhibits the cell division cycle & DHFR expression



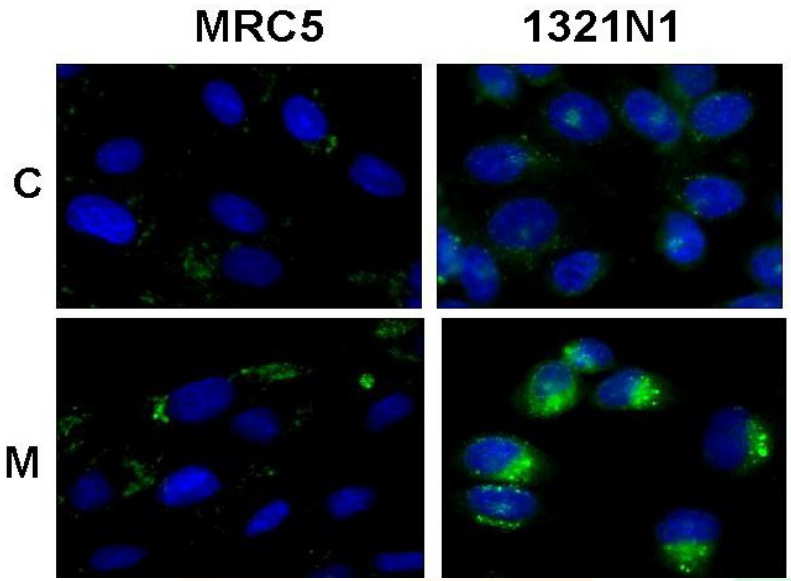




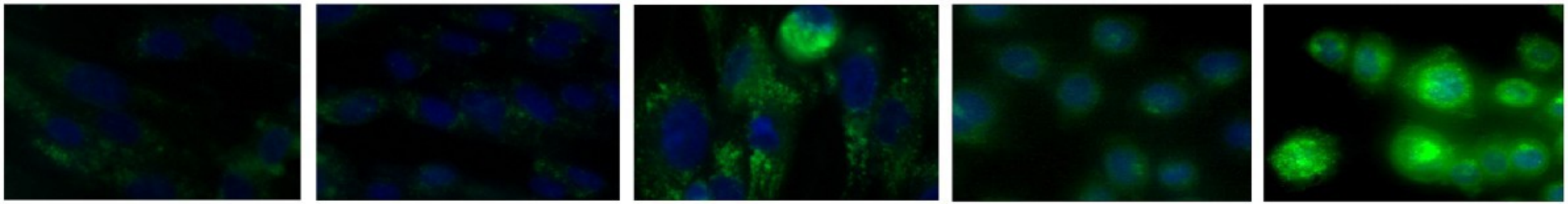


PKC translocation to the cell membrane (i.e., activation) induces overexpression of the CDKIs $p21^{Cip1}$ and $p27^{Kip1}$, and a concomitant reduction of cyclins and CDKs that causes RB hypophosphorylation and knockdown of E2F-1 and **DHFR**. The net result is induction of **cell cycle arrest**.

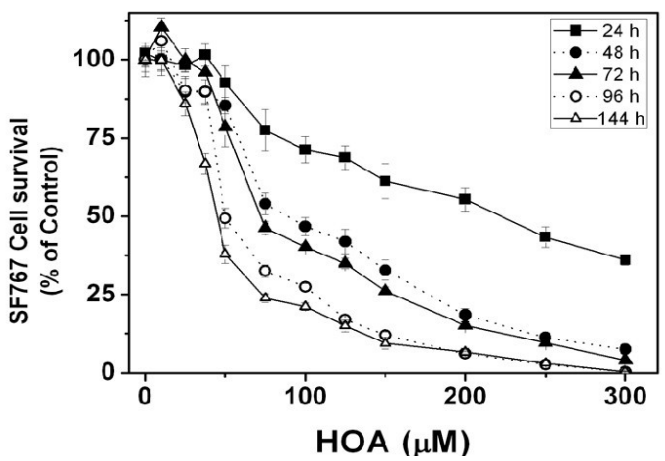
Minerval induces AUTOPHAGY in Glioma cells but not in normal cells

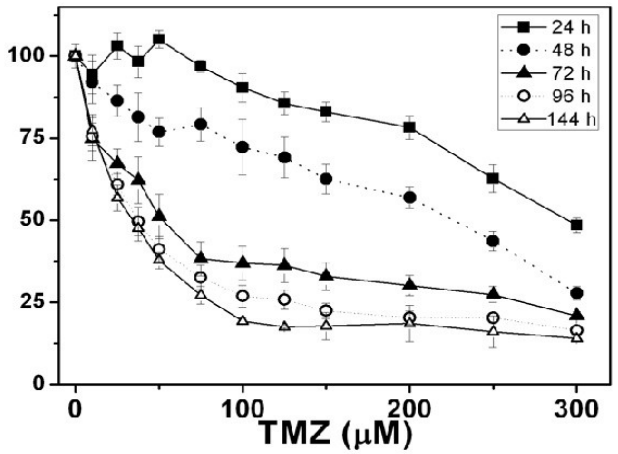


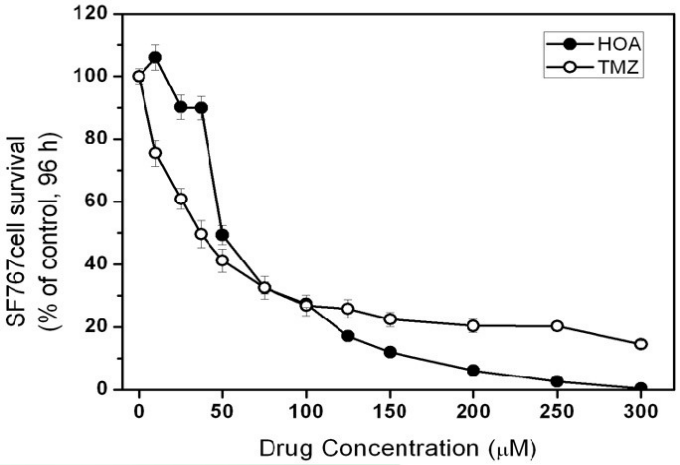
Minerval (2OHOA; M) selectively induced autophagy in human glioma 1321N1 cells (top) and SF767 cells (bottom) but not normal (MRC-5) cells. Fluorescence of lysosome/autophagosome labelled with Lysosensor in MRC-5 cells in the presence or absence (bottom left) of 2OHOA or palmitic acid (Pal), a known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

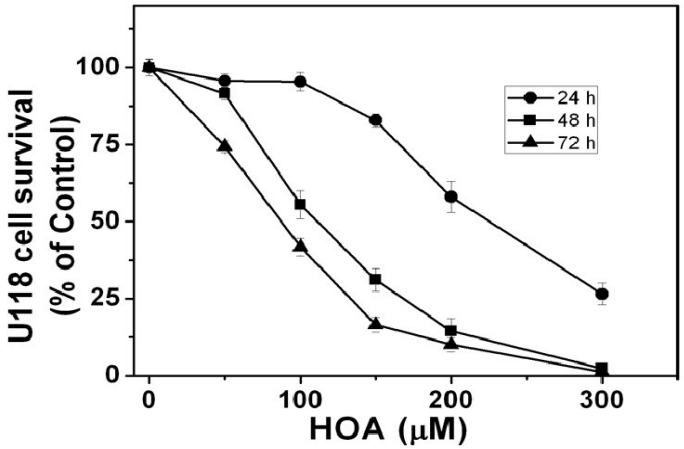


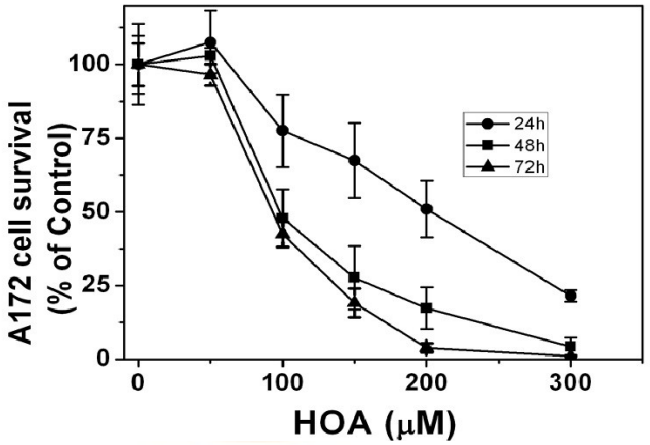
Efficacy of Minerval in cellular models

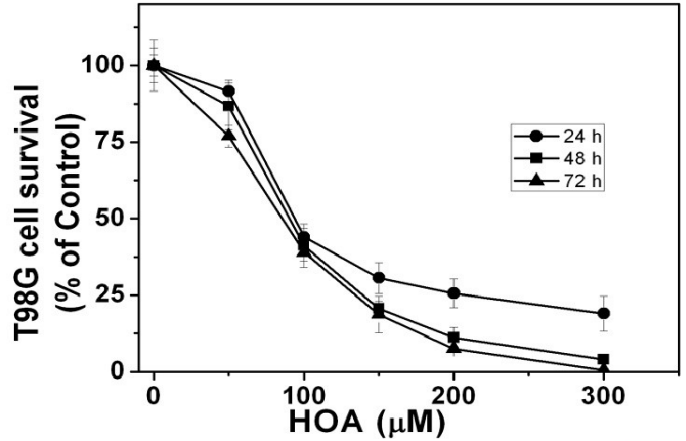






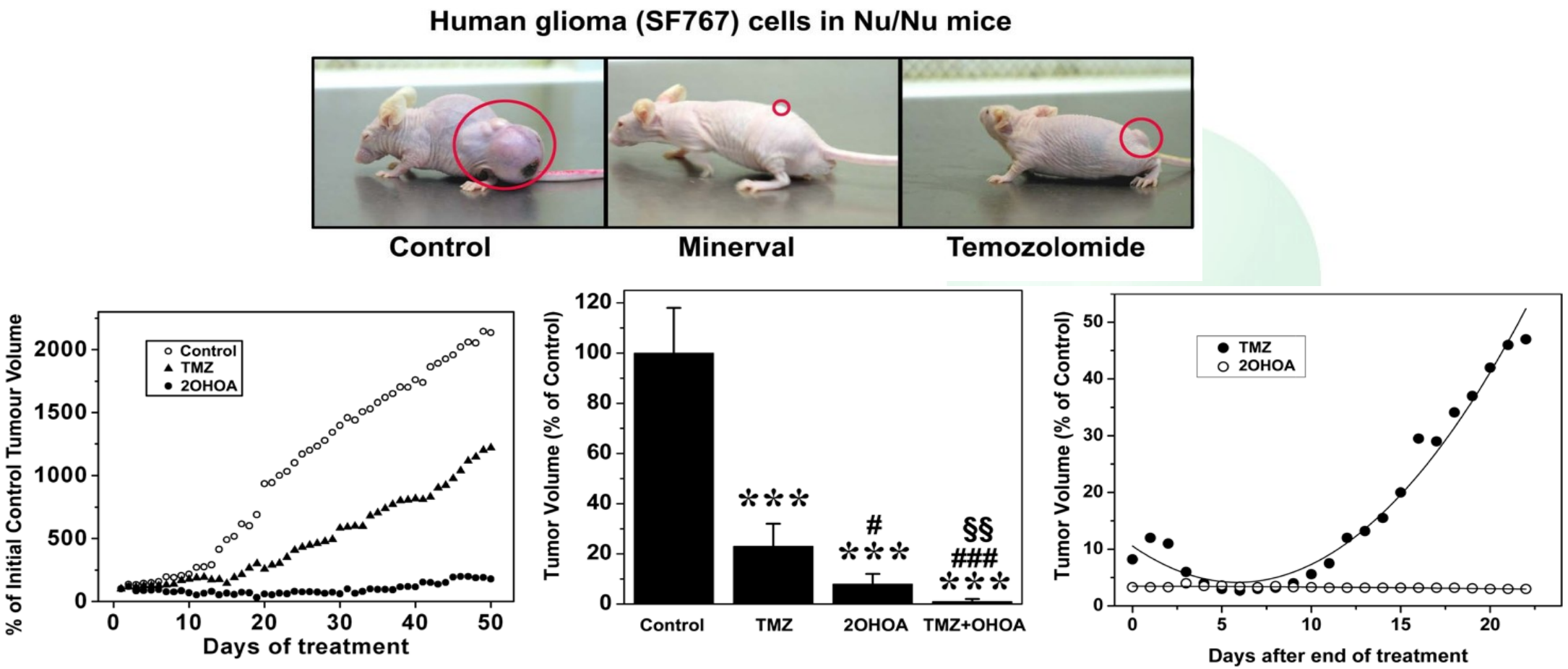






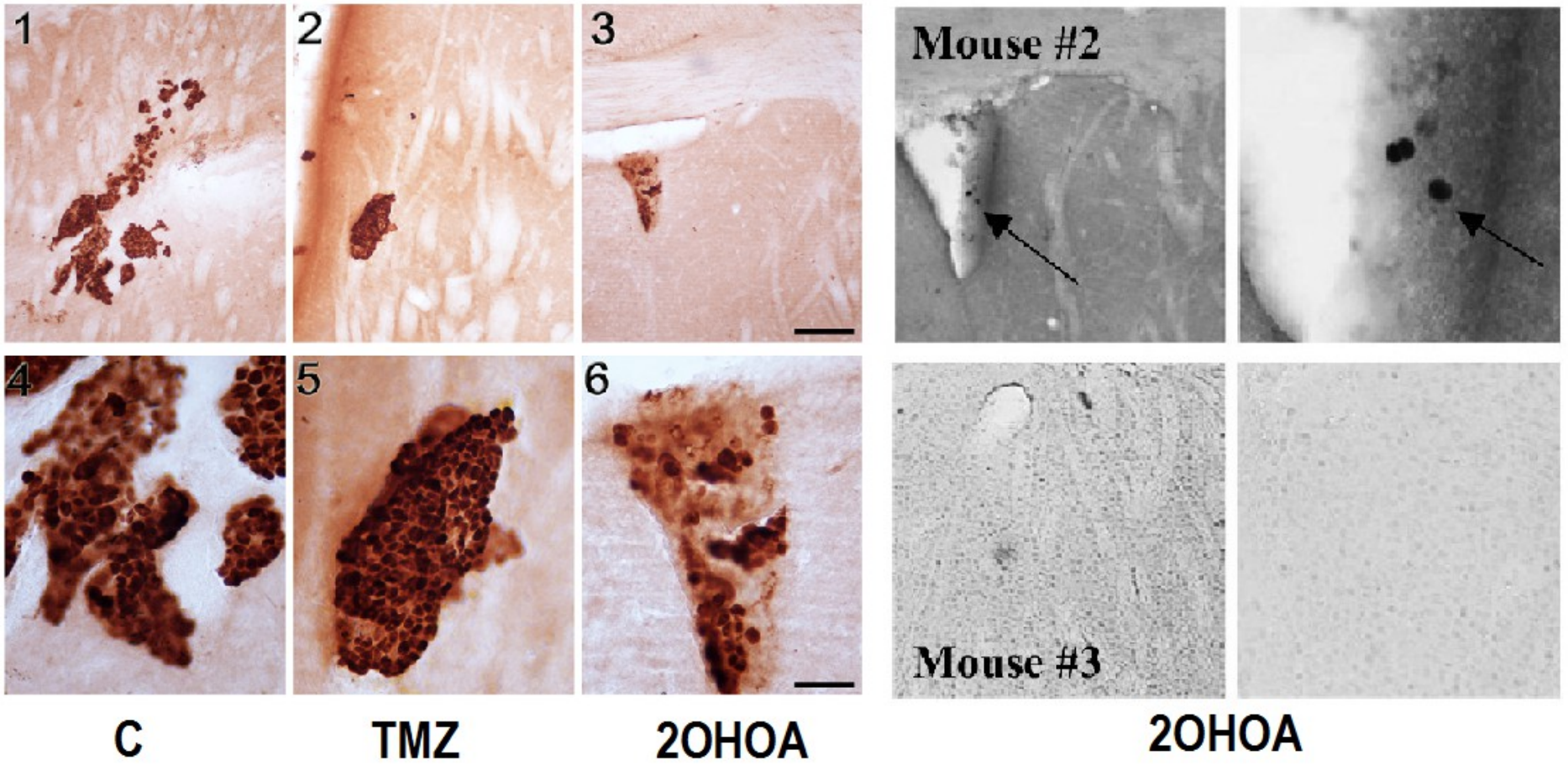
Minerval (HOA) is able to inhibit, in a time and concentration dependent manner, the growth of several human glioma cell lines (SF767, U118, A172, T98G). In SF767 cell line, Minerval clearly demonstrates a superior efficacy than temozolomide, which is not able to kill all cancer cells at 300 microM

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



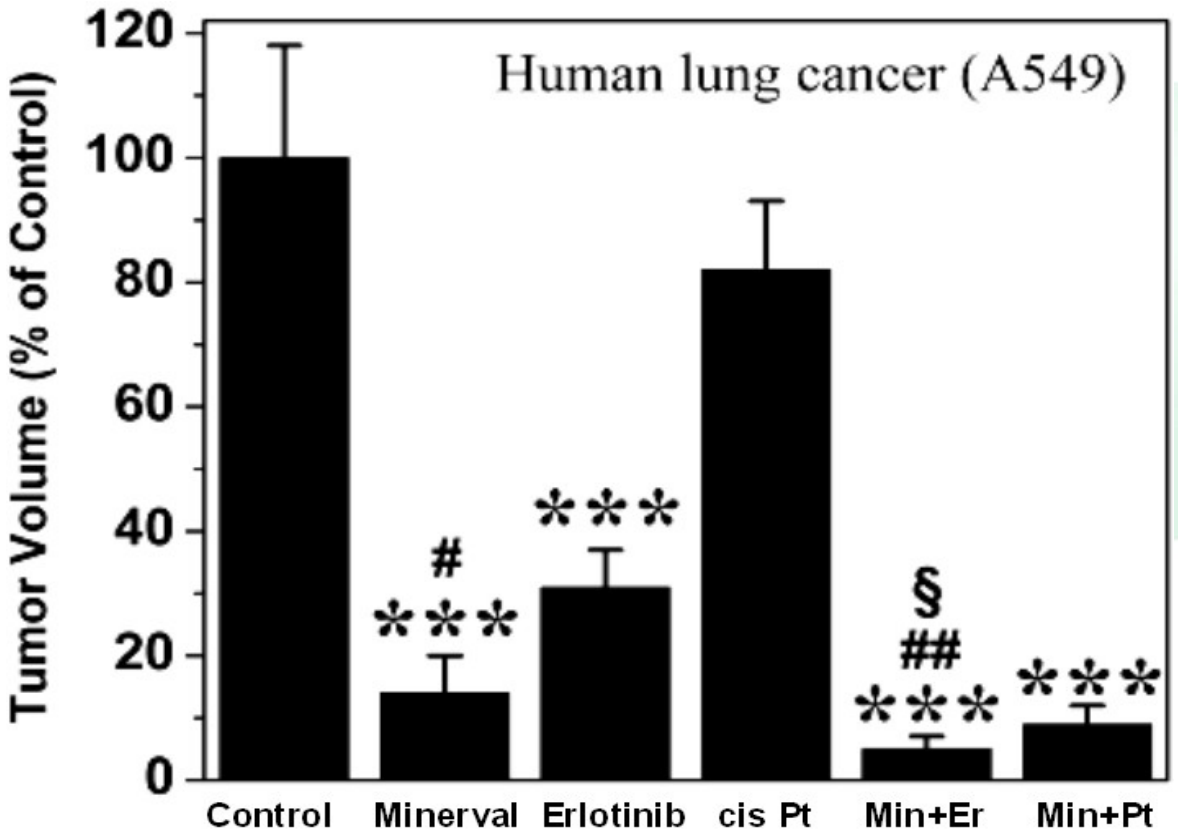
Minerval (2OHOA, OHOA) has demonstrated a potent anticancer effect in xenograft animal models, clearly outperforming temozolomide (TMZ) in 50 days treatment (bottom left). Combinatory regime with TMZ showed strong synergistic results after 60 days treatment (bottom middle). Moreover, animals treated with Minerval do not show tumour relapse after treatment termination, as it happens with animals administered with TMZ (bottom right)

Minerval crosses the BBR. Anti-cancer effect in an orthotopic model



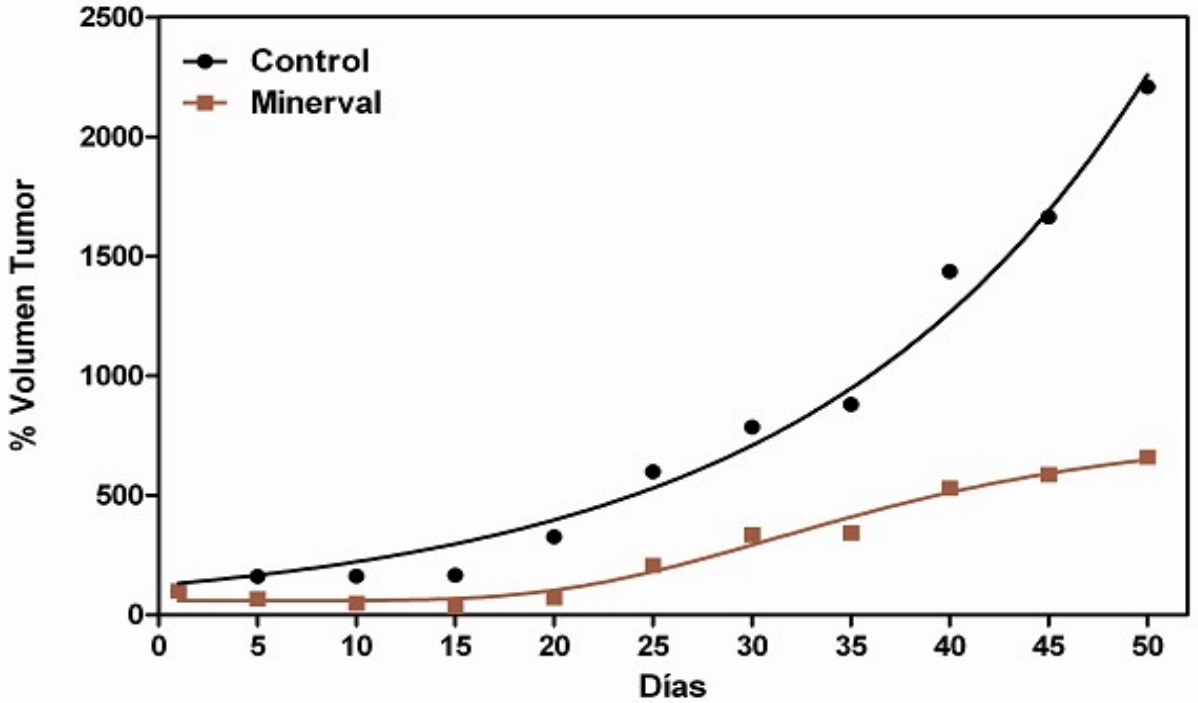
Immunocytochemical analysis of brains from nude mice inoculated with human glioma cells (SF767) and treated (p.o.) during 42 days with vehicle (control), Minerval or temozolomide (TMZ) Bars: 200 mm (1, 2, 3) and 50 mm (4, 5, 6)
In mouse #2 and mouse # 3 (treated with 2OHOA) almost/no traces of tumour were found (right panels)

IN VIVO EFFECT OF MINERVAL ON HUMAN LUNG TUMOURS (A549)

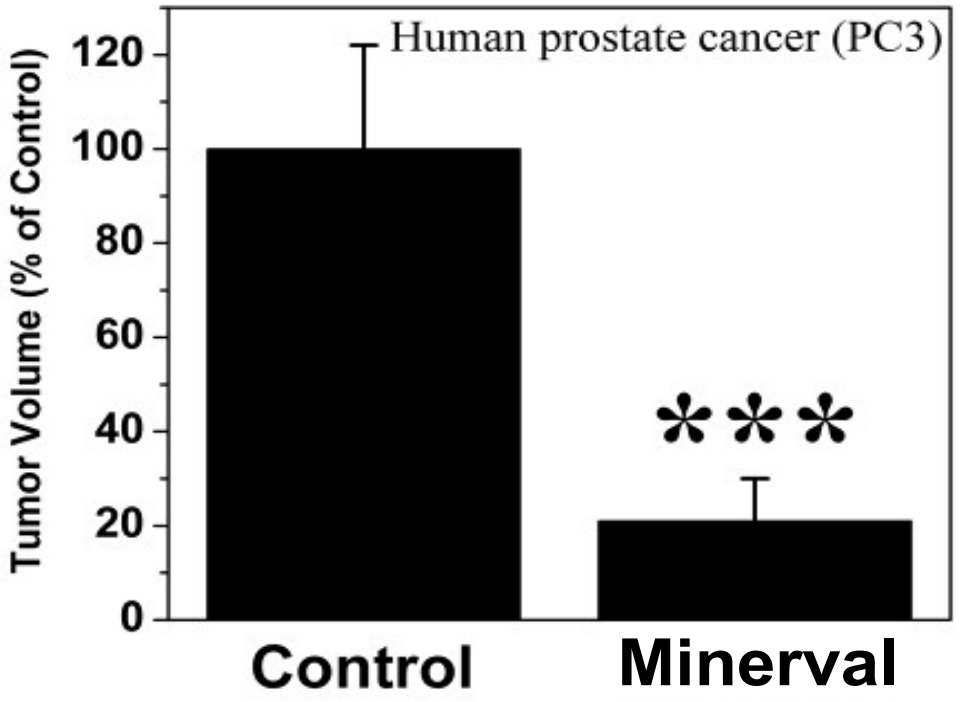


Comparative effect of Minerval (Min), Erlotinib (Er) & Cis Platinum (cis Pt /Pt) in mice xenograft models of Lung Cancer

IN VIVO EFFECT OF MINERVAL ON HUMAN PROSTATE CELLS (PC3)

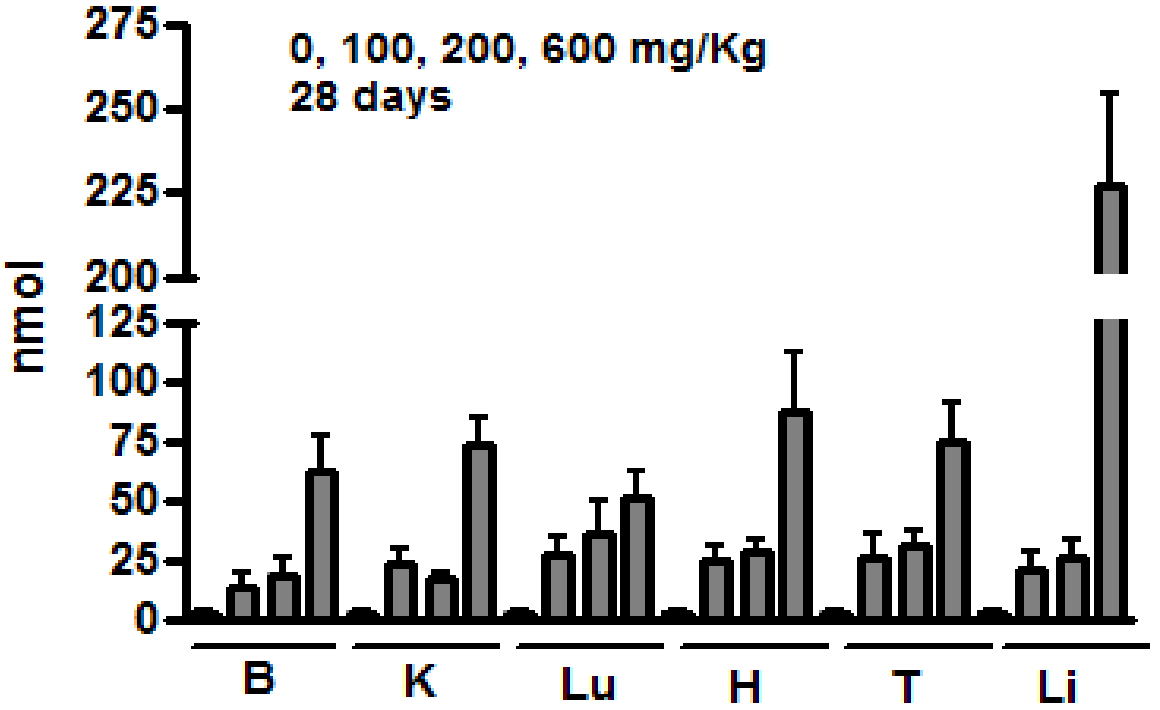


Effect of Minerval in mice xenograft models of PROSTATE CANCER tumours



[^{3}H]2OHOA distribution in nude mice

2OHOA accumulation in organs



Mice infected with SF767 cells were treated with various doses of Minerval (2OHOA) (0-600 mg/kg) for 28 days and their levels were measured in various organs by liquid scintilliation.
B, brain; K, kidney; Lu, lungs; H, heart; T, tumor; Li, liver.

Current status: PI/II Clinical Study with Minerval

MIN-001-1203: "*A phase I/IIa open-label dose escalation study of Minerval in subjects with advanced solid tumors including malignant glioma*".

Top leading European KOL and **investigational sites** involved: **Johann de Bono** (Royal Marsden Hospital, London), **Roger Stupp** (University Hospital, Zurich), **Jordi Rodon** (Vall d'Hebron Institute of Oncology, Barcelona), **Herbie Newell** and **Ruth Plummer** (Northern Institute for Cancer Research, Newcastle)

Part A. Dose escalating study. Up to 30 patients. 21-day treatments. Glioma and other solid tumors (lung, pancreas…)

Part B. Exploratory study. Up to 20 patients in two groups. 21-day treatments. 1st group with glioma patients. 2nd group with biopsiable solid-tumors patients for biomarker evaluation.

Biomarkers: **SM**, **DHFR** and **GFAP** (glioma only) + **Imaging** (FLT/PET, MRS), miRNA,...

CTA approved in Dec. 2012 by the MHRA (UK) & by the AEMPS (Spain) in Apr. 2013. First patients enrolled expected in May 2013

Minerval: chemistry

API

Obtained by chemical synthesis (GMP) through a simple and scalable process

Solid form, stable <25ºC (up to 24 months) and at -20ºC (up to 48 months). Stable for at least 7 days at 40ºC/75% RH --> can be stored and shipped ambient

CTM / Drug Product

Formulation developed for the first oncology trial: simple blend of powder in bottle for reconstitution with water into oral suspension. Powder formulation stable <25ºC for at least 9 months.

The final main pharmaceutical form will most likely be sachets/capsules for oral intake.

Strong global IP position

Inventions protected by **4 global Patent Families** covering the therapeutic and nutraceutical applications of MLT-based molecules and structural analogues

Main Patent for Minerval issued in all major markets, including **Europe**, **USA**, **Japan, China**, **Russia, Mexico...**

Freedom to Operate (FTO) available for cancer indications of Minerval

Minerval: Pitfalls & Risks

- Validation of a novel anti-cancer drug target!!
- Novel MOA
- "Harvard effect"
- Clinical & Financial risk
- too nice to be true!!
- ...

Lipopharma & Minerval today:

- **PI/II trials** in **glioma** & **solid tumors** started in 2013. CTA already approved in the UK and in Spain.
- **Orphan Drug** status granted in EU (glioma) in October 2011
- **7+ M Euro** already raised in equity / grants (2007-2012)
- Looking for **partnering/investing** opportunities with leader global oncology players for completion of clinical development in oncology

AWARD WINNER

EVC

EUROPEAN VENTURE CONTEST
SEMI-FINAL HEALTHCARE
24 OCT 2012 | AARHUS

Thank you!

Lipopharma

Ctra. Valldemossa, Km. 7,4. ParcBIT. Edif. Disset. 2º C-8. E07121 – Palma de Mallorca. Spain

☎+34 971 439 886 | info@lipopharma.com www.lipopharma.com