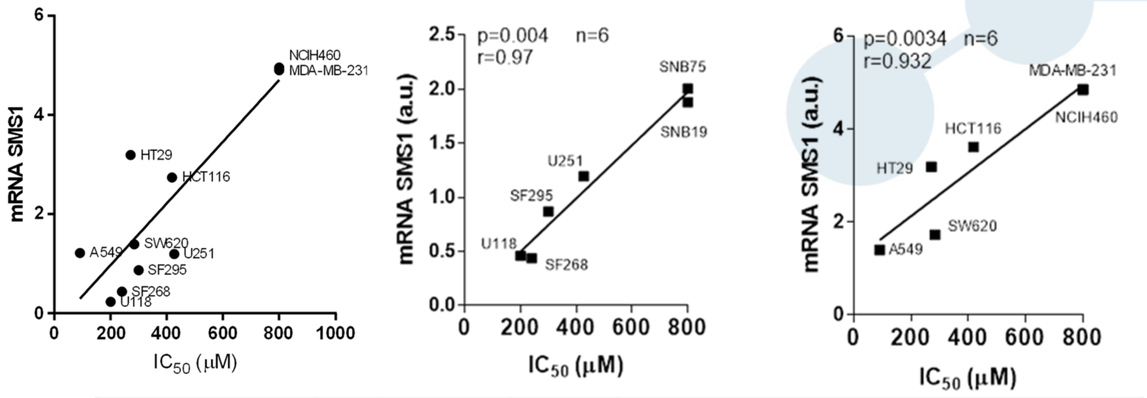
LP561A1 (2OHOA), A BREAKTHROUGH IN CANCER

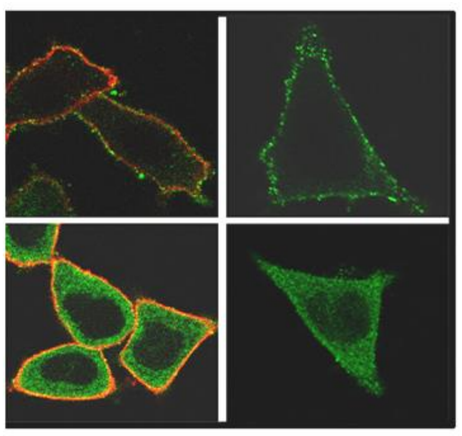
MECHANISM OF ACTION

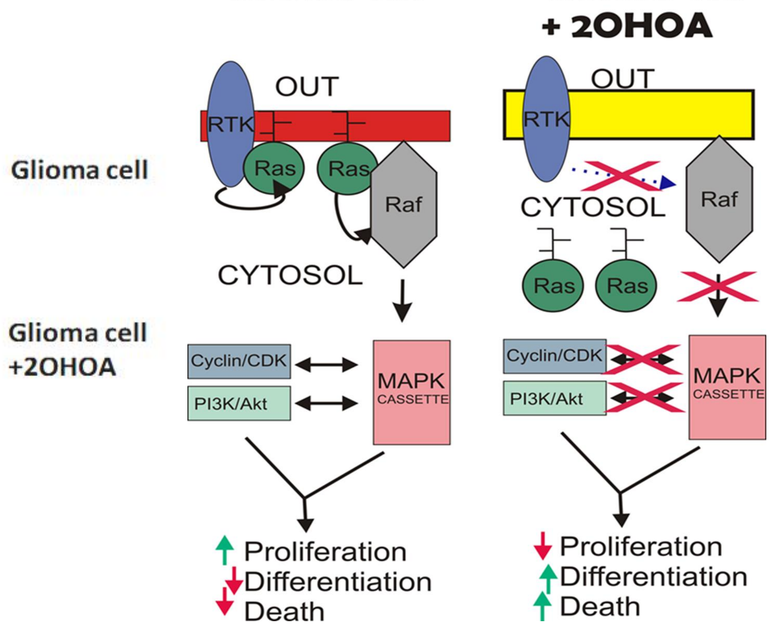
2OHOA ⇨ **+SMS1** ⇨ **(+)SM/PC (+)SM/Cer** ⇨ **(-) KRas nanoclustering & signalling** ⇨ **(-) MAPKs/PI3K/Akt/mTOR signalling** **(+) Immune system activation** ⇨ **Selective Cancer cell death**

2OHOA is an orally bioavailable synthetic derivative of oleic acid that crosses the Blood Brain Barrier and activates **sphingomyelin synthase 1** (**SMS1**), a key enzyme that catalyses the reversible conversion of PC, PE or PS into **SM** and DAG, leading to an increased **SM/PC** ratio and the formation of new lipid species as 2-OHOA-PC in the cancer cell membrane. This impairs **Ras nanoclustering** and **translocates K-Ras** from the cell plasma membrane to cytosolic cell membranes (e.g. Golgi membranes) and consequently **inhibits Ras-associated proliferative signalling pathways,** including MAPKs, Notch, PKC or P13K/Akt/mTOR. In addition, the modulation of **Cer/SM** and **PC/SM ratios** have been reported to **boost the immune system** against the tumour

Right. The anti-tumoral effect of 2OHOA (IC_{50}) on a variety of cancer cell lines strongly correlates (r2=0,9) with basal levels of **SMS1** mRNA expression: left all cancer; middle glioma; right other cancer (non-glioma).







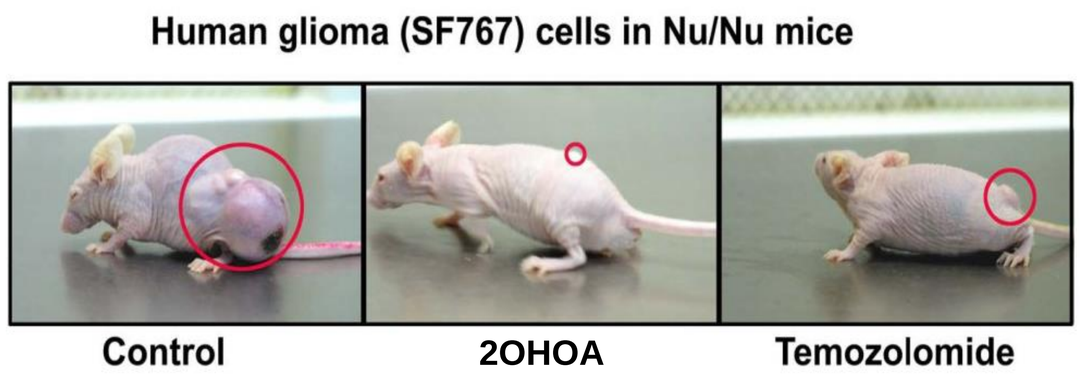
The activation of SMS1 accumulates **sphingomyelin-2OHOA** in the cell membrane, translocating **K-Ras** to non-active cytosolic domains in human glioma U118 cells (**top left**). As a consequence K/H-Ras mislocalizes from its active domain in the plasma membrane inhibiting its nanoclustering and Ras-dependent proliferation pathways (like Ras/MAPK, Pi3K/AKT/mTOR or PKC/Cyclin CDK), causing cell cycle arrest followed by selective death of cancer cells (**top right**).

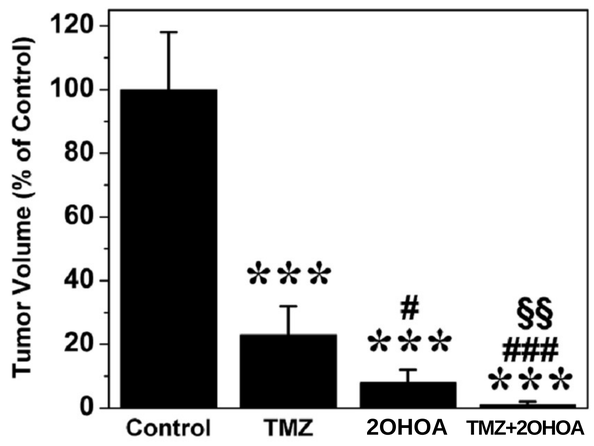
ID	N	lor	pval	name
GO:0002460	14	-0.606	0.025	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains
GO:0042098	11	-0.661	0.022	T cell proliferation
GO:0002449	13	-0.647	0.022	lymphocyte mediated immunity
GO:0050670	12	-0.664	0.021	regulation of lymphocyte proliferation
GO:0070663	12	-0.664	0.021	regulation of leukocyte proliferation
GO:0046651	15	-0.624	0.014	lymphocyte proliferation
GO:0070661	15	-0.624	0.014	leukocyte proliferation

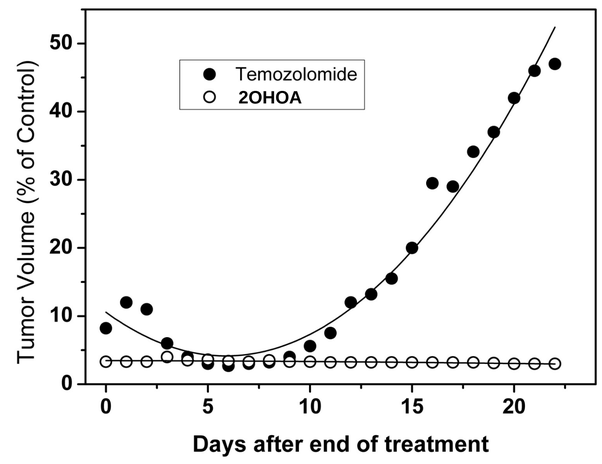
2OHOA activates immune system in cancer patients. Gene ontology functions determined by the expression of miRNAs analysis performed in patient samples (Clinical Study MIN-001-1203) demonstrated an activation of the antitumoral immune response in cancer patients (negative lor values indicate a blocking activity of miRNA inhibitory effects)

EFFICACY IN PRECLINICAL STUDIES

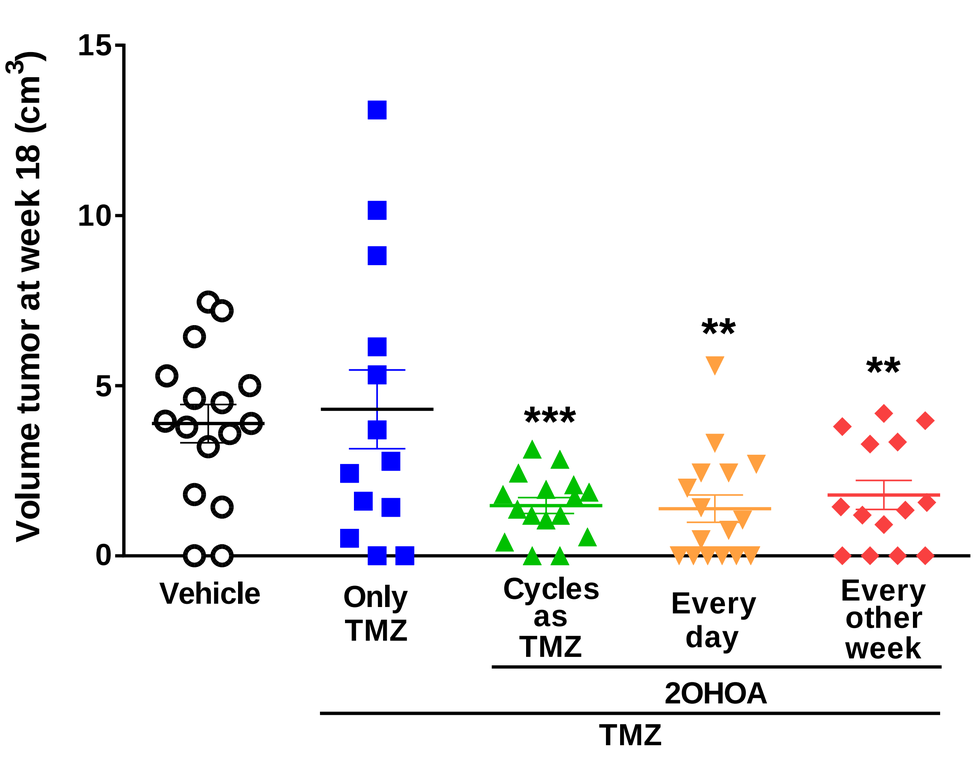
2OHOA has greater efficacy than temozolomide (TMZ) and no tumour relapse was observed after treatment termination



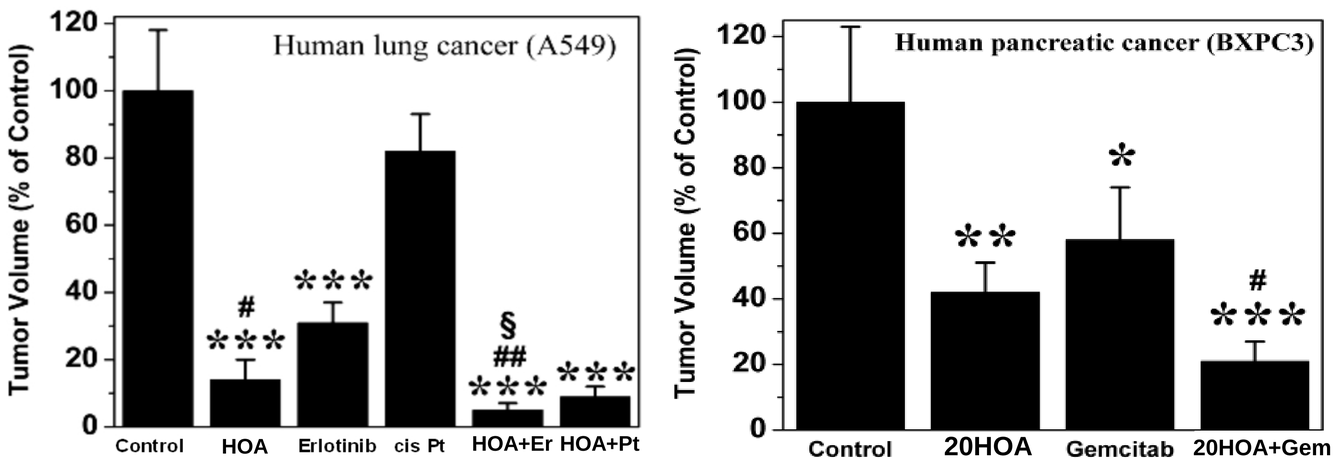




Top. 2OHOA has demonstrated a marked anticancer effect in xenograft animal models with SF767, outperforming temozolomide (TMZ) in 50-day treatment (upper right). Combinatory regime with TMZ showed strong synergistic results after 60-day treatment. Animals treated with 2OHOA do not show tumour relapse after treatment termination, as it happens with animals treated with TMZ.



Efficacy of combinatory treatment of 2OHOA plus TMZ in a xenograft mice model with non-metilated cells partially non-responsive to TMZ (U118MG), exploring different posology regimes of 2OHOA+TMZ.



2OHOA (HOA) efficacy in mice xenograft models of human lung cancer (A549) and pancreatic cancer (BXPC3), all with low SMS1 levels.

Supported by:

LP561A1 (2OHOA), A BREAKTHROUGH IN CANCER

MIN-001-1203: "A phase I/IIa open-label dose escalation study of 2-OHOA in subjects with advanced solid tumors including malignant glioma"

Study design / results

Part A. Dose escalating study. 32 patients (pts) in 7 cohorts. 21-day cycles.

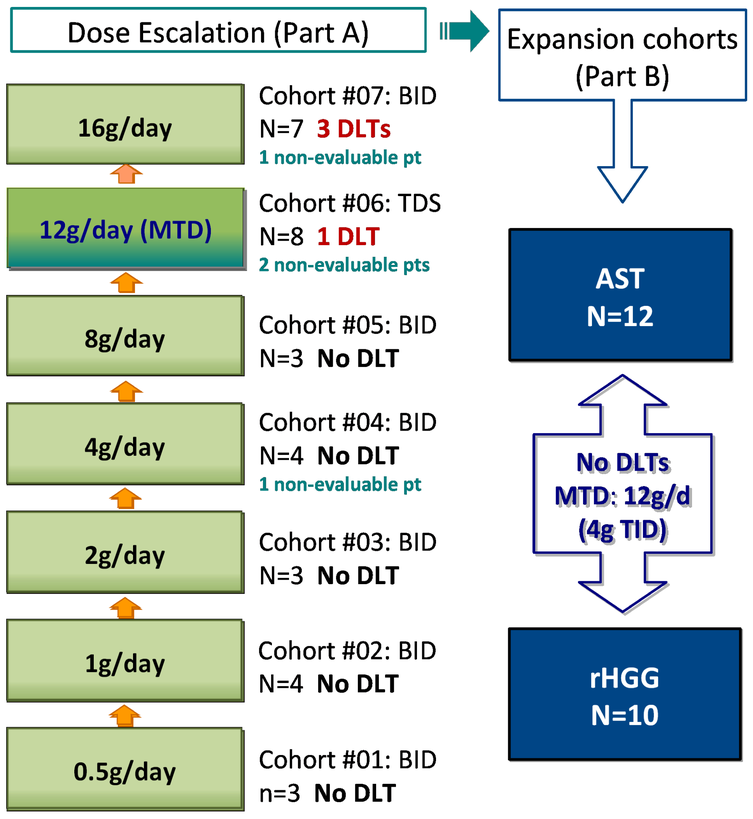
Part B. Exploratory study. 22 pts in 2 cohorts. 21-day cycles. Cohort #30: 12 glioma pts. Cohort #40: 10 biopsiable Advanced Solid-Tumours (AST) pts for biomarker evaluation.

Objectives

Dose-escalation, multicentre phase I clinical trial of 2-OHOA in advanced solid tumours including recurrent malignant glioma

- **Primary objective**: To determine safety, tolerability and recommended phase 2 dose of of 2-OHOA
- **Secondary objectives**: To determine pharmacokinetics (PK) and pharmacodynamics (PD) profile and preliminary anti-tumoral activity
- **Exploratory objectives**:
  - *To evaluate the effect of 2-OHOA on glial fibrillary acidic protein (**GFAP**) in glioma pts*
  - *To study **miRNA** as a potential response biomarker*

Main results



Part A highlights (*completed*):

» 7 cohorts completed. 32 pts (28 ≥1cycle) treated

» No relevant **safety issues** (only GI effects (diarrhea, nausea,..) in some pts at the higher doses)

» **MTD** established as 12g/day (4g TID)

Part B highlights (*completed*):

» 22 pts treated with 12g/day.

» **No DLTs**. No safety/tolerability issues

» **MTD** confirmed as 12g/day (4g TID)

Anti-tumour activity

Encouraging sings of efficacy in heavily pre-treated recurrent AST, specially in glioblastoma (**rGBM**) pts:

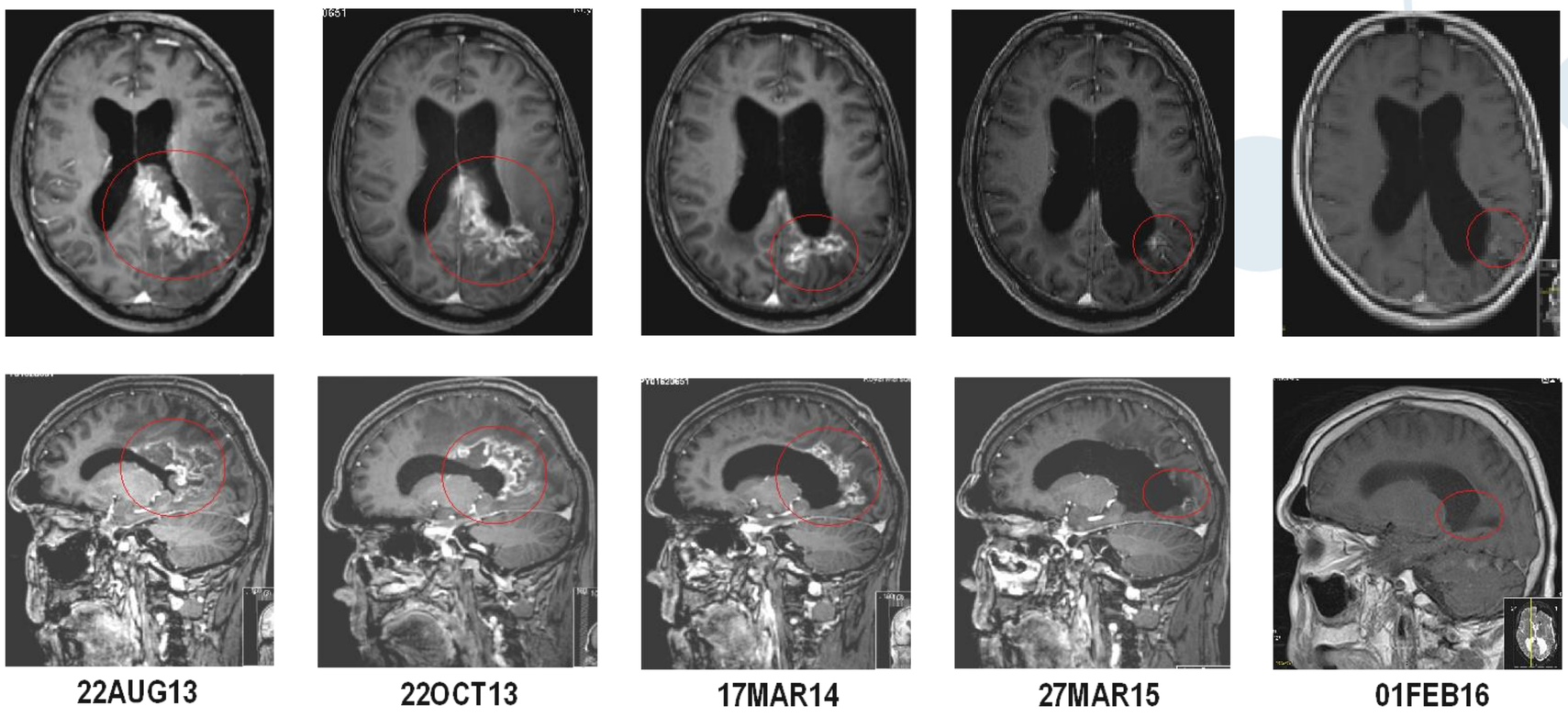
» **Clinical benefit** by RANO/RECIST in **13 pts** (8 with malignant glioma)

» 50% (4/8) of **rGBM** pts treated for ≥2 cycles had objective clinical benefit for at least 6 months (m.)

- » **1 rGBM → PR for almost 3 years**
- » **1 oligodendroglioma → SD for 9 m.**
- » **1 rGBM → SD for 7 m.**
- » **2 rGBM → SD for 6 m.**
- » **1 mesothelioma → SD for 10 m.**
- » **1 colon ADK → SD for 3 m.**
- » **1 biliay duct ADK → SD for 5 m.**

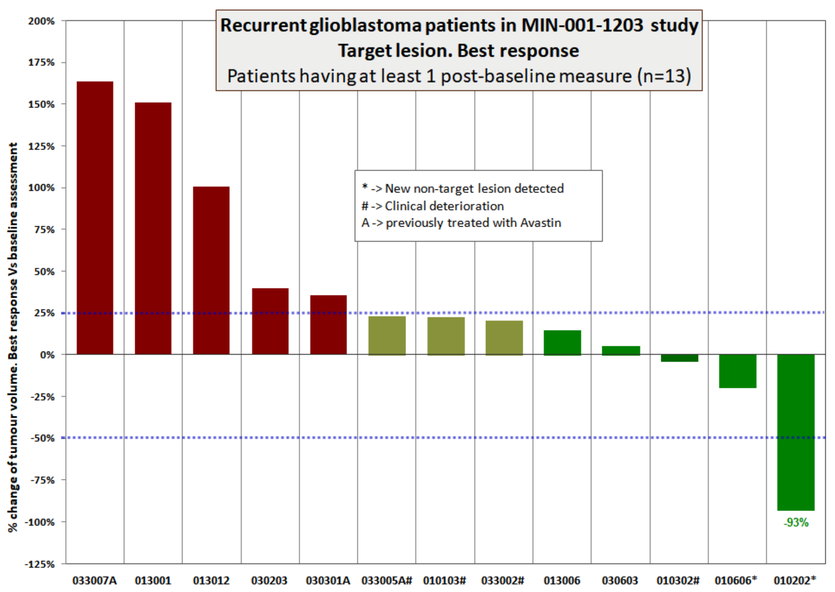
Case study 1: response in GBM patient 010202 (54y old male)

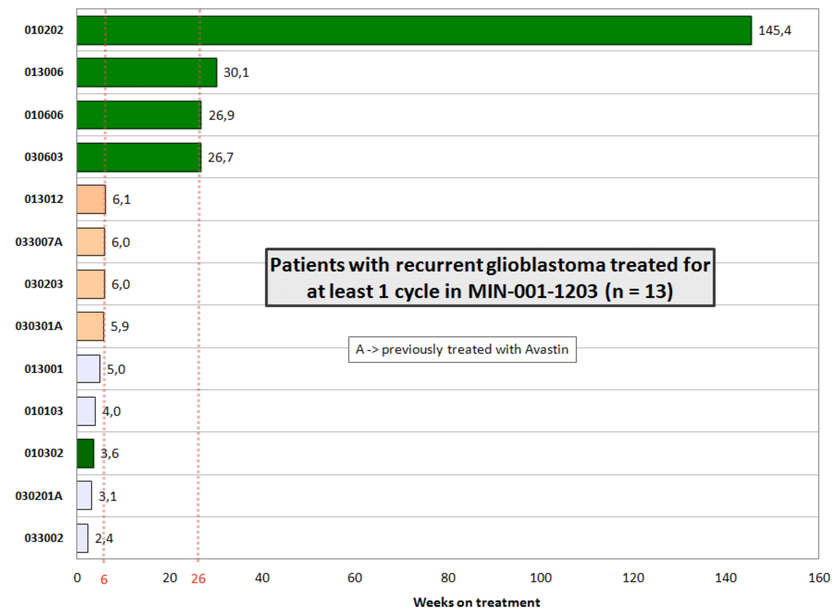
- Apr 2012: Partial debulking surgery followed by radical chemo-radiotherapy
- Aug-Sep 2012: Adjuvant Temozolomide, with PD after 3 cycles
- Nov-Feb 2013: PCV chemotherapy with PD after 4 cycles
- Aug 2013: enrolled in MIN-001-1203 trial (2nd cohort, 1g/day BID)



Top. Pt 010202. Partial Response (PR) by RANO (**tumour shrinkage** of **93%** on primary lesion lasting for almost **3 years**)

2-OHOA in recurrent GBM patients in MIN-001-1203 PI study





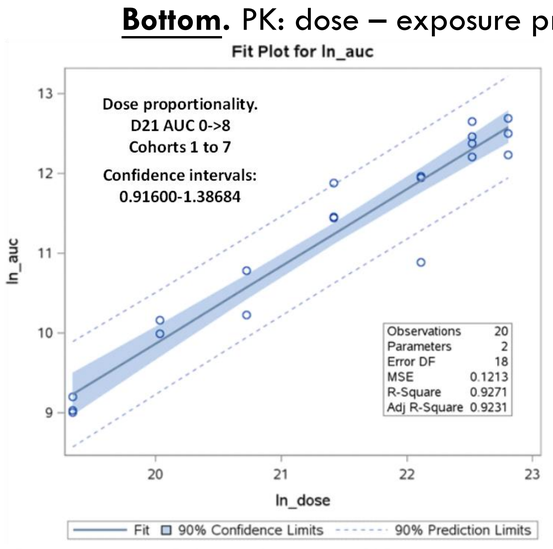
➔ **13** rGBM patients completed at least 1 cycle of treatment with 2OHOA. 5/**13 rGBM** patients treated had overall clinical benefit.

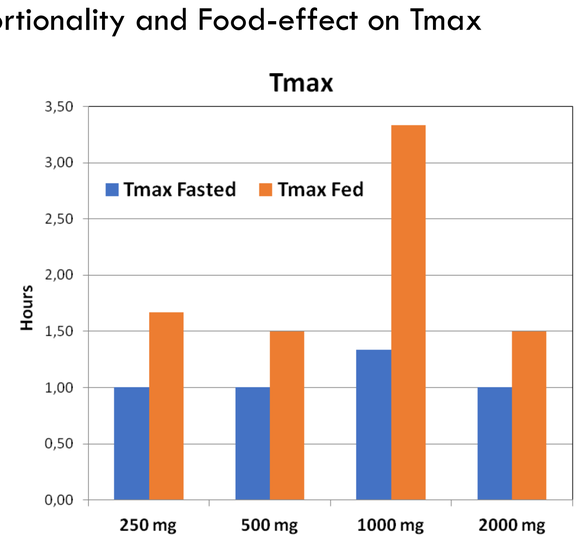
➔ **8** rGBM patients **completed at least 2 cycles of treatment** and had their planned reassessment scan at 6 weeks. **4** of them (**50%**) had **clinical benefit** by RANO **for at least 6 months.**

➔ All rGBM pts with clinical benefit had previously received 2 or more lines of treatment without bevacizumab.

➔ Overall, more that 50% of rGBM pts treated (7/13) had **radiological outcome** within the range of SD or PR (best response), but in 2 cases clinical deterioration was confirmed and therefore they were assessed to have progressive disease (PD)

Bottom. PK: dose – exposure proportionality and Food-effect on Tmax





Pharmacokinetic profile

2OHOA was quantifiable in all dose levels and **Cmax** was reached 1 hour post-dose (fasted).
Food effect was not relevant. Fed and fasted bioavailability of 2OHOA were comparable, although food caused a non-clinically significant delay in Tmax from 1 hour to 2-4 hours.
Systemic exposure of 2OHOA increased in **proportion to dose** following repeated BID administration. After repeated BID dosing, systemic exposure of 2OHOA, increased between 1- and 2,0-fold from first dose on Day 1 to last dose on Day 21. Median **effective half-life**, calculated based on accumulation ratio, was 11h [min. 5h; max. 19h]

Pharmacodynamics

GFAP. A reduction of GFAP levels in plasma from rHGG patients after 8 days of treatment observed in more than 80% of patients analyzed. Average reduction in GFAP levels in the whole set of patients was 20% (n=15)

miRNA. At least 3 miRNA found to be differentially expressed in response to 2OHOA treatment (n=22).

Further biomarker testing (DHFR, SM, SMS1, SMS2, genomic evaluation,...) ongoing in samples obtained from MIN-001-1203 study

MIN-001-1203. Safety summary

✔21 Serious Adverse Events (**SAE**) reported in all cases assessed as "**not related**" or "**unlikely related**" to the study drug

✔No **treatment-related deaths**

✔**Dose limiting toxicities (DLTs)** were Grade 3 diarrhoea (n=3) and vomiting (n=1) despite medical optimization

✔Of the **202** drug-related Adverse Events (AE) reported in the 54 patients treated in the study, **97%** (195/202) were **grade 1-2** and **3%** (7/202) were **grade 3**

✔The majority of the adverse events related to the treatment were of **gastrointestinal** (GI) nature, including all Grade 3 AEs

✔No safety or tolerability issues reported in the **safety expansion cohorts**: 22 patients treated with 12000mg/day, several of them treated for >6 months

Supported by: