# IN VITRO ANTINEOPLASTIC EFFECTS ON HUMAN GLIOMA CELLS OF LACOSAMIDE AND BRIVARACETAM

A. Salmaggi°, A. Rizzo*, S. Donzelli#, V. Girgenti*, A. Sacconi#, C. Vasco*, G. Blandino#, M. Maschio^, E. Ciusani*,

*Department of Clinical Pathology and Medical Genetics - Foundation IRCCS Neurological Institute Carlo Besta - Milan;°Department of Neurosciences-Stroke Unit - A. Manzoni Hospital-Lecco;
^Center for tumor-related epilepsy, Area of Supporting Care - Regina Elena National Cancer Institute - Rome; #Oncogenomics Traslational Unit - Regina Elena National Cancer Institute - Rome

**BACKGROUND -** Epilepsy is a frequent complication in patients with glioma, however the use of antiepileptic drugs is not always sufficient for seizure control. The MRPs (multidrug resistance proteins) and P-gp, (P-glicoprotein) were found to be overexpressed in brain tissue of patients with drug-resistant epilepsy, suggesting their involvement in the clearance of antiepileptic drugs.
In addition to the anticonvulsant mechanism, for some antiepileptic drug a cytotoxic effect has been previously suggested (Eyal S et al., Epilepsia, 2004).
**AIM –** Aim of the work was to evaluate possible *in vitro* cytotoxic effects of two new-generation antiepileptic drug on human glioma cell lines. Expression of MRPs was also evaluated on the same cells before and after *in vitro* exposure to sub-cytotoxic drug concentration.

## METHODS

CYTOTOXICITY ASSAY
We studied the *in vitro* effects of two new-generation antiepileptic drugs, brivaracetam (BRV) and lacosamide (LCM), on human glioma cell lines (U87MG, SW1783 and T98G), by cell proliferation assay (MTS) at 72h of treatment. Inhibitory concentrations (IC) were calculated from the regression line that correlates percentage of growth inhibition and drug concentrations calculated as:

100-(100*average n. cells [0-2500µM] /n. cell basal level)

APOPTOSIS
Evaluation of apoptotic cells was performed in untreated and treated cells ($IC_{20}$ BRV or $IC_{20}$ LCM for 24-48-72h) using Annexin V binding assay - Annexin V-FITC Apoptosis detection kit- by flow citometry (FacsVantage SE, Becton Dickinson, CA, USA) . Data are expressed as percentage of apoptotic cells.
MICRORNA EXPRESSION PROFILE
Total RNA from U87MG cells, treated with BRV or LCM ($IC_{20}$) fo 24, 48 and 72h, was purified, labelled and hybridized on "Human miRNA Microarray V19" slides (Agilent), that contain probes for 2000 human microRNA. Scanning and image analysis were performed using the scanner "Agilent DNA Microarray Scanner (P/NG2565BA)". "Feature Extraction Software" (versione 10.5) was used to extract data.
CELL CYCLE
Cells (8X105) were plated in triplicate and cultured for 24 h. LCM and BRV $IC_{20}$ were then added for 72h. At each time-point cells were harvested and fixed in ethanol 80% at 4°C for 30 min, washed and stained with PI 50 µg/ml in PBS overnight at 4°C. DNA content was evaluated using flow-cytometry.
MIGRATION CELL STUDY
Migratory ability of U87MG cells transfected with mimic control or mimic miR-107 was evaluated by plating cells in serum-free medium on transwell with 8µm porous membrane (BD falcon) using medium additioned with 10% serum as chemoattractant. After 16h membranes were cut from the insert and migrated cells were visualized by DAPI staining and counted.

## RESULTS

CYTOTOXICITY ASSAY AND APOPTOSIS
Results show a cytotoxic effect of lacosamide (Pearson correlation index p <0.00001, fig.1A) and brivaracetam (Pearson correlation index p<0.00001, fig.1B) for U87MG at 72h of treatment. Cytotoxicity was confirmed by preliminary data on other two glioma cell lines SW1783 and T98G (Pearson correlation index p<0.05, fig.1C-F). Antiproliferative effect of BRV and LCM on U87MG cell line does not seem to correlate with an apoptotic mechanism (data not shown).

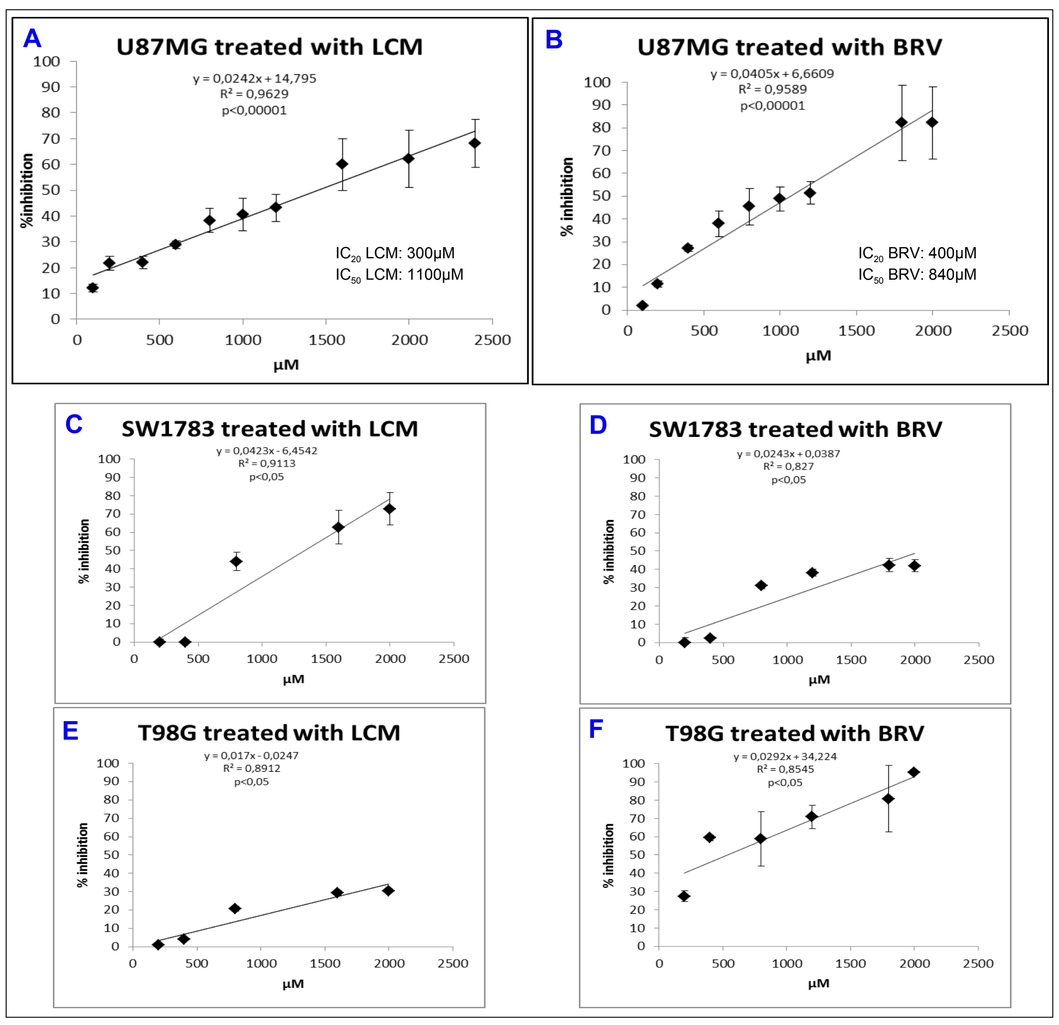


**Fig. 1**

CELL MIGRATION STUDY
U87MG cells migration assay following induction of mir-107 expression shows microRNA inhibitory effect on migration ability in glioblastoma cells (fig. 3A). This effect is also reflected in a decreased expression of the main proteins involved in migration such as EGFR, N-cadherin and cyclin-D1 (Fig. 3B).

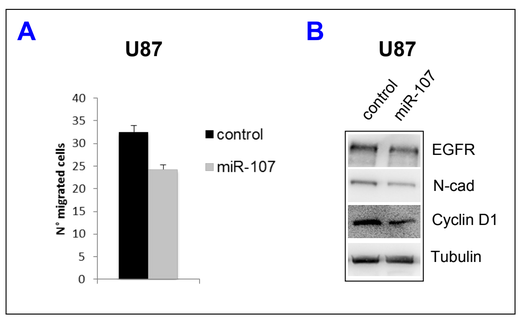


**Fig. 3**

MICRORNA EXPRESSION PROFILE
The analysis of microRNA expression profiles on U87MG cells enable us to identify microRNA modulated by BRV (fig. 2A) and LCM (fig. 2B). In detail, we identify 37 microRNA modulated by BRV and 30 miR by LCM. We validate our results with RealTime PCR on miR-107 and miR-195, that were induced by both treatments (fig. 3C). In literature there are several evidences that demonstrate an anticancer role of both microRNAs that act by inhibiting both the proliferation that the migration of cancer cells (fig. 2D).

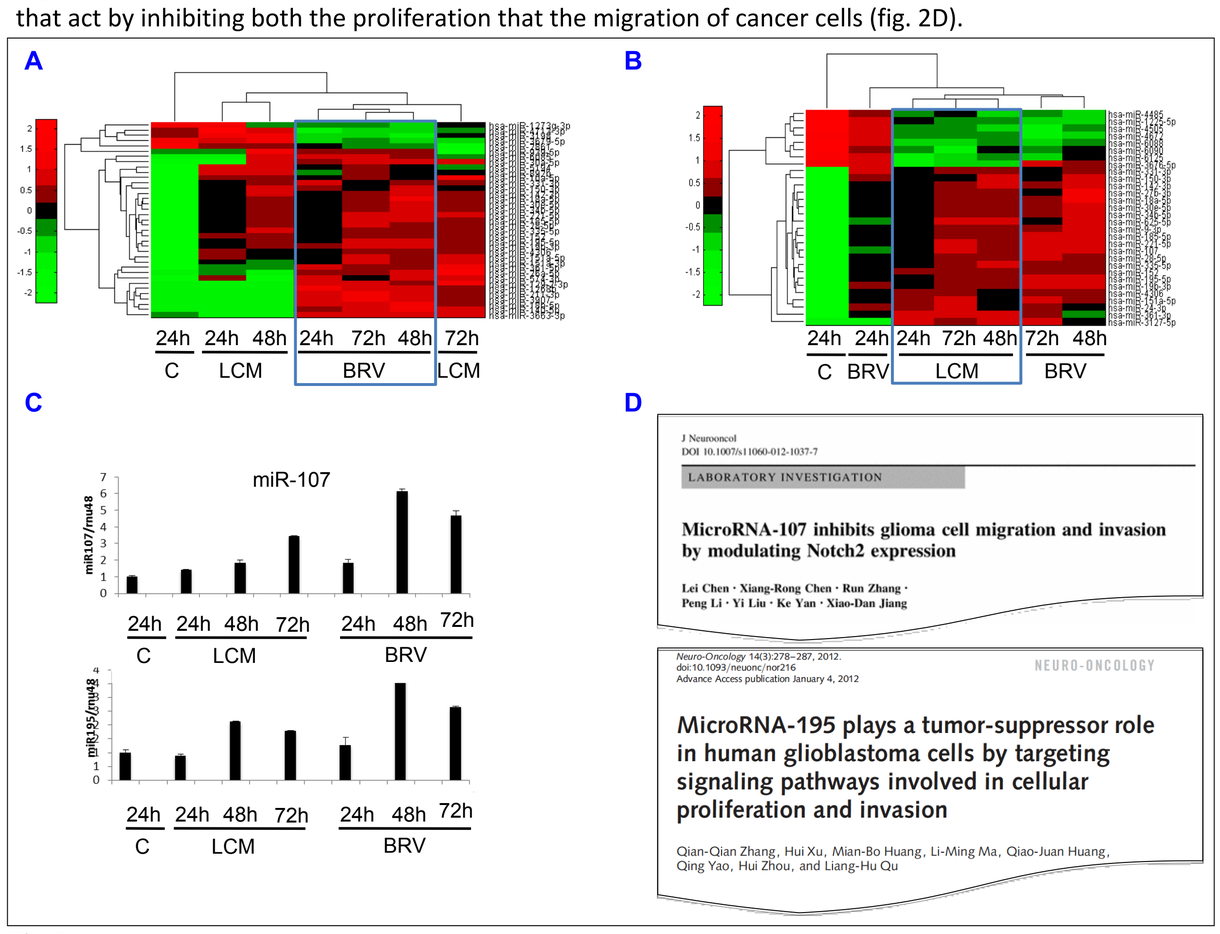


**Fig. 3**

Upon BRV and LCM IC20 treatments in U87MG cells, we observed an increase in p21 protein levels that suggested an anti-proliferative effect for these two drugs (Fig.4).

Overexpression of miR-195-5p induced a significant increase in the percentage of cells in G0/G1 phase of the cell cycle after 48 h from transfection and a concomitant decrease in G2/M (Fig.5).

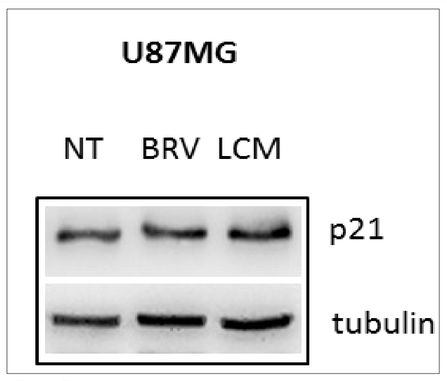


**Fig. 4**

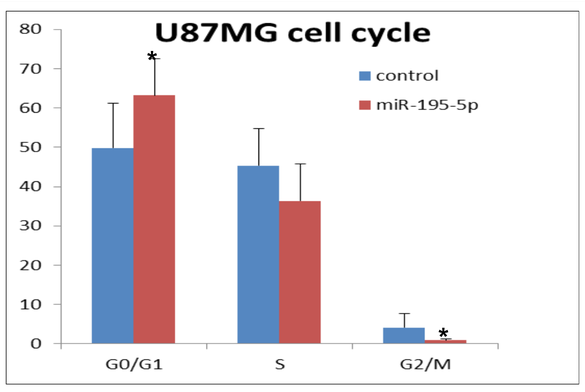


**Fig. 5**

## CONCLUSIONS

- Brivaracetam and lacosamide showed a cytotoxic effect on glioma cell lines U87MG, SW1783, T98G *in vitro*.
- The cytotoxic effect of BRV and LCM on U87MG does not seem to be related to induction of apoptosis.
- U87 cells treatement with BRV and LCM cause modulation of different microRNA like miR-107 and miR-195, which may partly explain the effect exerted by the two drugs. Moreover our data suggest a possible involvement of miR-107 in inhibition of cell migration.
- Our data suggest that, even at low doses ($IC_{20}$), the two drugs exert a role in blocking cell cycle progression of glioma cells possibly trough up-regulation of miR-195-5p and increased cyclin-dependent kinase inhibitor p21 expression.

## REFERENCES

•Baltes S et al. Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by humanand mouse P-glycoprotein. Neuropharmacology. 2007
•Beyreuther BK et al. Lacosamide: a review of preclinical properties. CNS Drug Rev. 2007
•Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the Ninth Eilat Conference (EILAT IX). Epilepsy Res. 2009
•Bialer M et al. Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI).Epilepsy Res. 2013
•Bobustuc GC et al.Levetiracetam enhances p53-mediated MGMT inhibition and sensitizes glioblastoma cells to temozolomide. Neuro Oncol. 2010
•Calatozzolo C et al. Expression of drug resistance proteins Pgp, MRP1, MRP3, MRP5 and GST-pi in human glioma. J Neurooncol. 2005
•Chen L et al. MicroRNA-107 inhibits U87 glioma stem cells growth and invasion. Cell Mol Neurobiol. 2013
•Chufan EE et al. Multiple transport-active binding sites are available for a single substrate on human P-glycoprotein(ABCB1). PLoS One. 2013
•Ciusani E et al.Valproic acid increases the in vitro effects of nitrosureas on human glioma cell lines.Oncol Res. 2007
•Kwan P et al. Drug-resistant epilepsy. N Engl J Med. 2011
•Kwiecińska P et al. Effects of valproic acid and levetiracetam on viability and cell cycle regulatory genes expression in the OVCAR-3 cell line. Pharmacol Rep. 2012
•Li XN et al. Valproic acid induces growth arrest, apoptosis, and senescence in medulloblastomas by increasing histone hyperacetylation and regulating expression of p21Cip1, CDK4, and CMYC. Mol Cancer Ther 2005
•Luna Tortós C et al. The Antiepileptic Drug Topiramate is a Substrate for Human P-glycoprotein but Not Multidrug Resistance Proteins. Pharm Res 2009
•Neuman MG, Nanau RM, Shekh-Ahmad T, Yagen B, Bialer M. Valproic acid derivatives signal for apoptosis and repair in vitro. Clin. Biochem. 2013
•Perucca E. What is the promise of new antiepileptic drugs in status epilepticus? Focus on brivaracetam, carisbamate, lacosamide, NS-1209, and topiramate. Epilepsia. 2009 Porojan D,
•Prakash O, Lukiw WJ, Peruzzi F, Reiss K, Musto AE. Gliomas and seizures. Med Hypotheses. 2012
•Stander M et al. Anticonvulsant drugs fail to modulate chemotherapyinduced cytotoxicity and growth inhibition of human malignant glioma cells. J Neurooncol 1998
•Zhang C et al. Potential role for human P-glycoprotein in the transport of lacosamide. Epilepsia. 2013
•Takai N et al., Human ovarian carcinoma cells: histone deacetylase inhibitors exhibit antiproliferative activity and potently induce apoptosis. Cancer. 2004 Dec 15;101(12):2760-70.
•Zhang QQ et al. MicroRNA-195 plays a tumor-suppressor role in human glioblastoma cells by targeting signaling pathways involved in cellular proliferation and invasion. Neuro Oncol. 2012 .
•Zhou Y et al. Histone deacetylase inhibitor, valproic acid, radiosensitizes the C6 glioma cell line in vitro. Oncol Lett. 2014 Jan;7(1):203-208. Epub 2013 Nov 7.
•Eyal S, Yagen B, Sobol E, Altschuler Y, Shmuel M, Bialer M. The activity of antiepileptic drugs as histone deacetylase inhibitors. Epilepsia 2004 Jul;45(7):737–744.