PTEROCARPANQUINONE LQB-118 INDUCES APOPTOSIS MEDIATED BY AKT AND ERK PATHWAYS IN GLIOBLASTOMA CELLS

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INTRODUCTION AND OBJECTIVE

Glioblastoma (GB) is the most common astrocytoma and a lethal human malignancy, with a median survival of 14 months. GB treatment is based on surgery followed by radiotherapy and adjuvant chemotherapy with temozolomide (TMZ). The development of new therapies is imperative for this disease. The purpose of the study was to evaluate the antitumoral activity of the new synthetic compound, LQB-118 and depth the understanding of the mechanism of action.

RESULTS

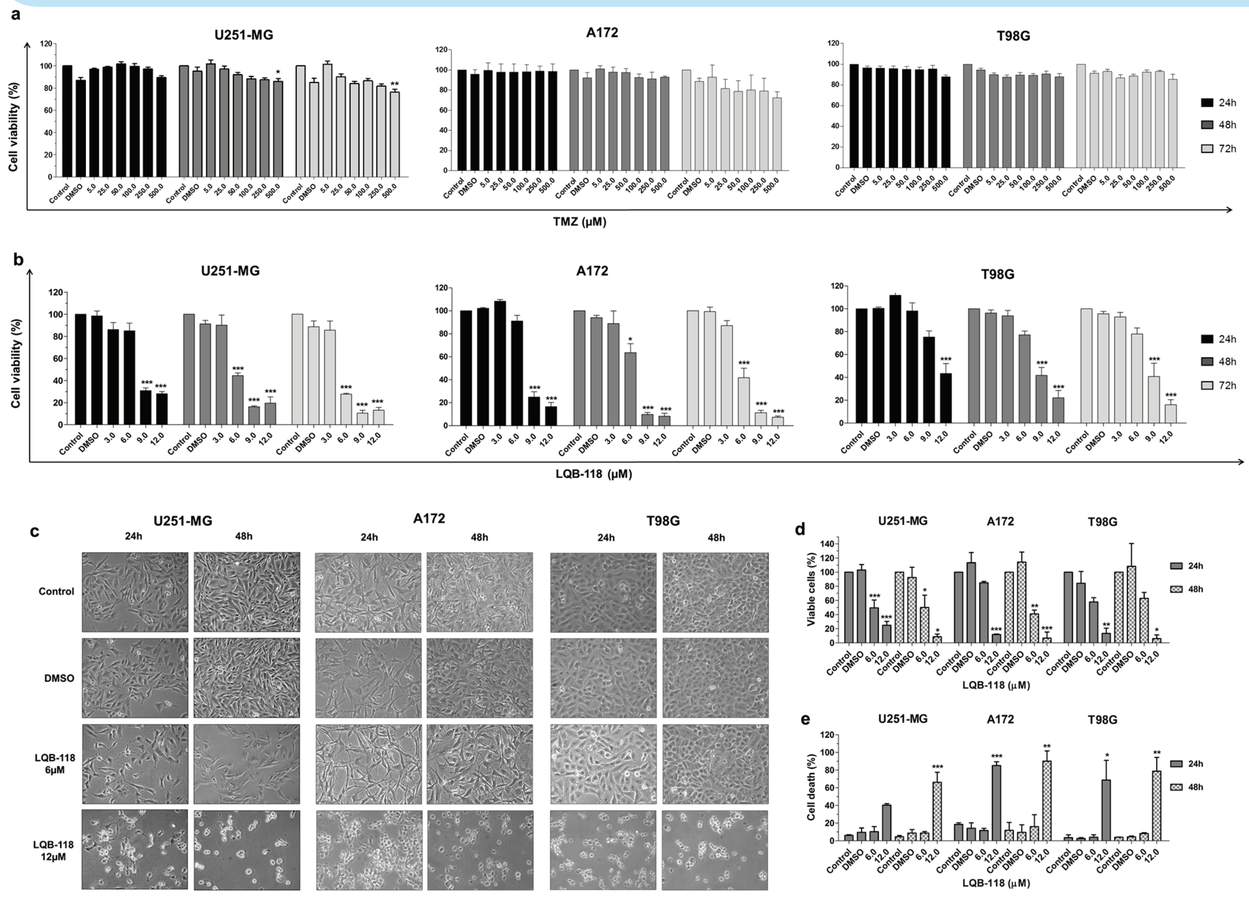

Figure 1. LQB-118 effect on cell viability and cell death. Percentage of U251-MG, A172 and T98G viable cells after treatment with increasing concentrations of temozolomide (TMZ) (a) and LQB-118 (b) evaluated for 24, 48 and 72 h. Contrast phase photomicrography showing morphological features observed after treatment with LQB-118 obtained in 10 times magnification (c). Percentage of cell viability (d) and cell death (e) evaluated by trypan blue exclusion assay after LQB-118 treatment. Mean of three independent experiments ± standard error. *p<0.05, p<0.01, ***p<0.001

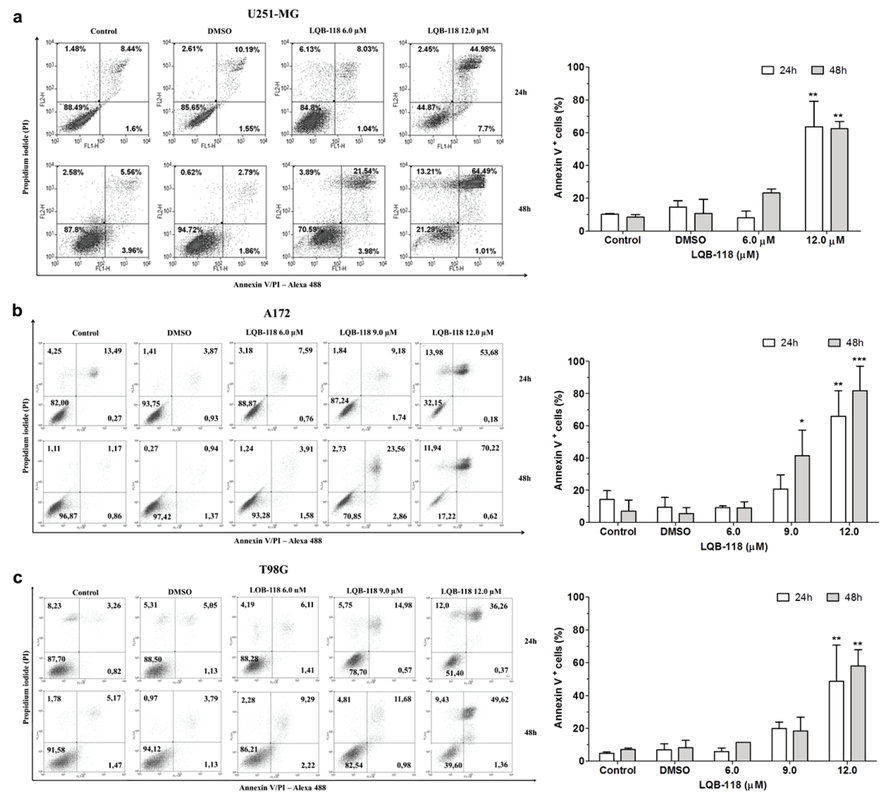

Figure 2. Apoptosis evaluated by annexin V/PI assay after LQB-118 treatment. U251-MG (a), A172 (b) and T98G cells were treated with LQB-118 for annexin V/PI labeling analysis by flow cytometry. Representatives dot plots (graphics in the left column) and bar graphics (in the right column) with mean of three independent experiments ± standard deviation (annexin positive cells = annexinV+/ PI– + annexinV+/ PI+) were plotted. *p<0.05,

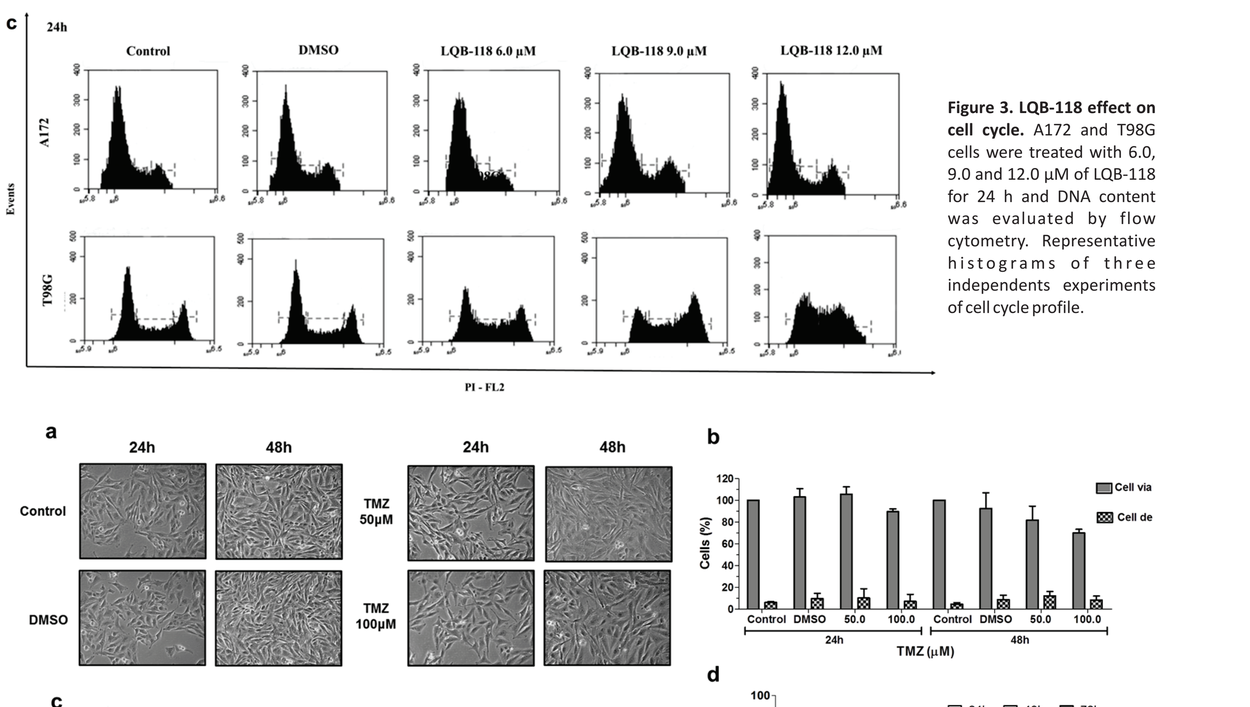

Figure 3. LQB-118 effect on cell cycle. A172 and T98G cells were treated with 6.0, 9.0 and 12.0 μM of LQB-118 for 24 h and DNA content was evaluated by flow cytometry. Representative histograms of three independents experiments of cell cycle profile.

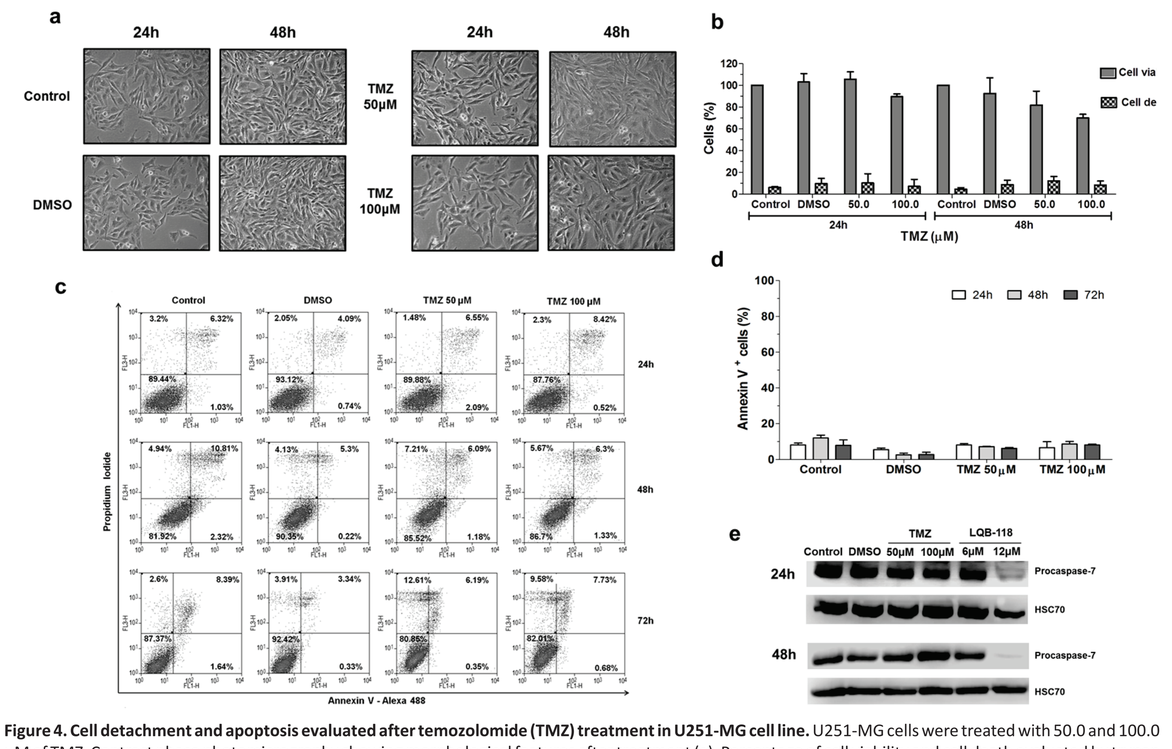

Figure 4. Cell detachment and apoptosis evaluated after temozolomide (TMZ) treatment in U251-MG cell line. U251-MG cells were treated with 50.0 and 100.0 μM of TMZ. Contrast phase photomicrography showing morphological features after treatment (a). Percentage of cell viability and cell death evaluated by trypan blue exclusion assay. Mean of three independent experiments ± standard deviation (b). Representatives dot plots of annexin V/PI labeling after TMZ treatment for 24, 48 and 72 h (c). Bars graphic with mean of three independent experiments ± standard deviation (annexin positive cells = annexinV+/ PI– + annexinV+/ PI+) (d). Procaspase-7 expression evaluated after treatment with 6.0 and 12.0 μM of LQB-118 or 50.0 and 100.0 μM of TMZ. HSC-70 (70kDa) expression was used as endogenous control. Figure representative of two independent experiments (e). TMZ= temozolomide

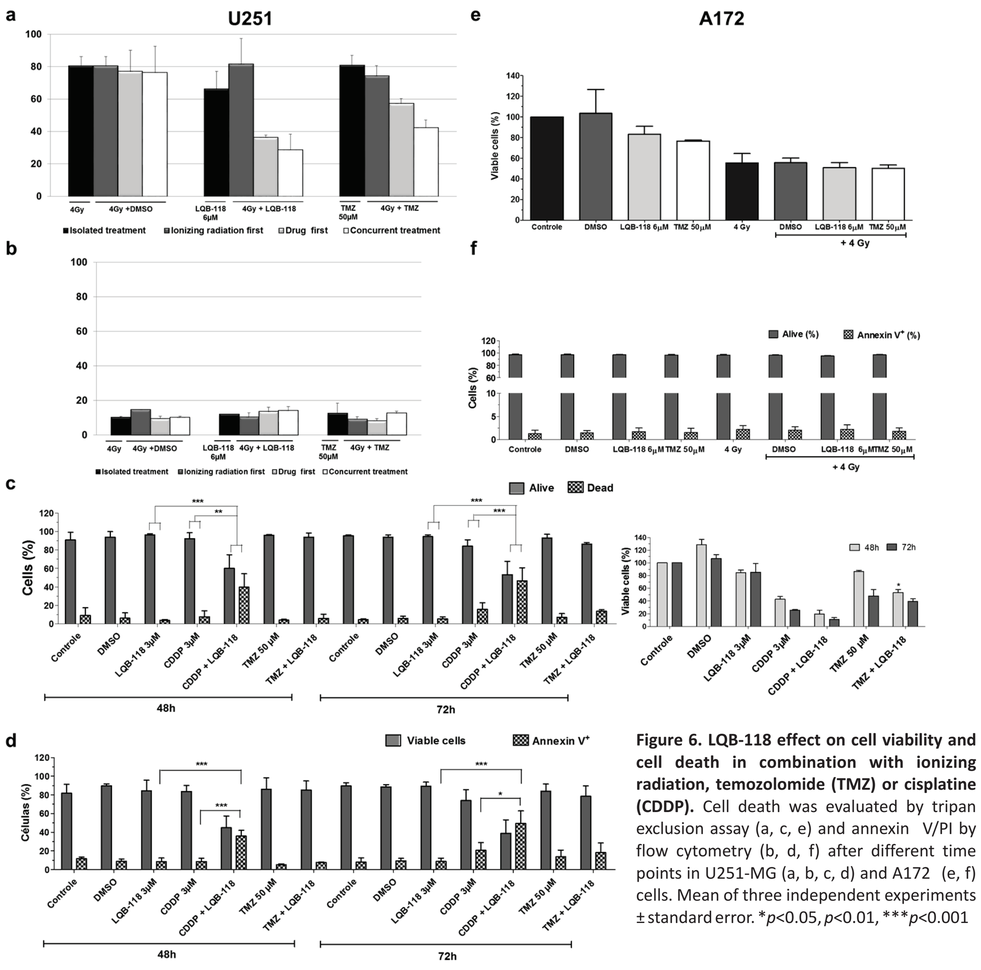

Figure 6. LQB-118 effect on cell viability and cell death in combination with ionizing radiation, temozolomide (TMZ) or cisplatine (CDDP). Cell death was evaluated by tripan exclusion assay (a, c, e) and annexin V/PI by flow cytometry (b, d, f) after different time points in U251-MG (a, b, c, d) and A172 (e, f) cells. Mean of three independent experiments ± standard error. *p<0.05, p<0.01, ***p<0.001

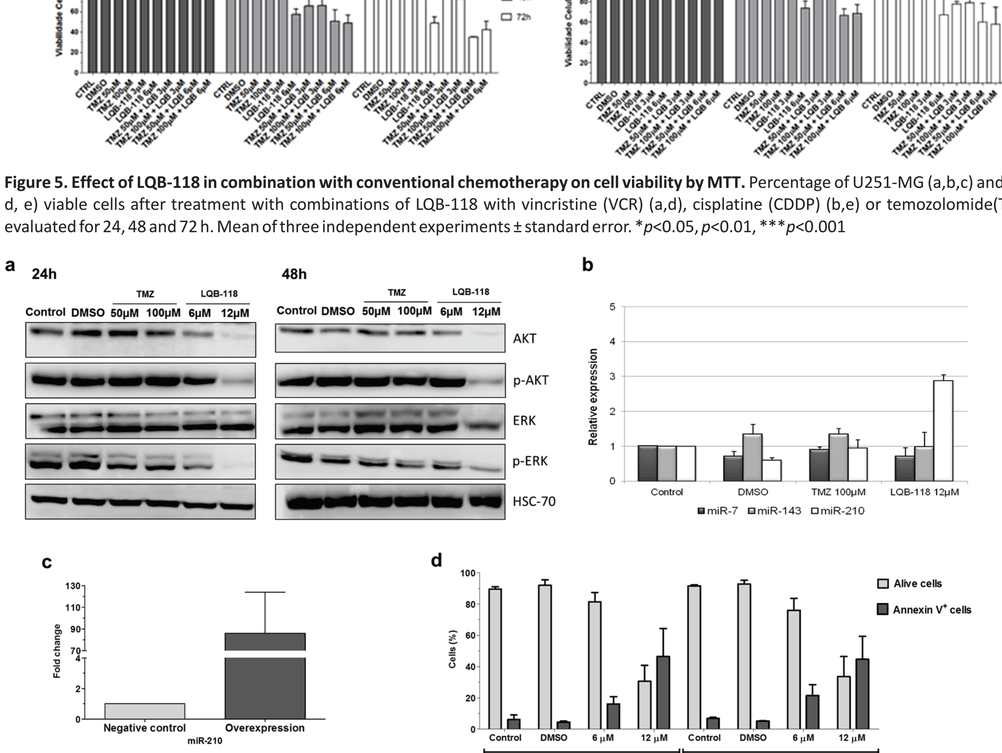

Figure 5. Effect of LQB-118 in combination with conventional chemotherapy on cell viability by MTT. Percentage of U251-MG (a,b,c) and A172 (c, d, e) viable cells after treatment with combinations of LQB-118 with vincristine (VCR) (a,d), cisplatine (CDDP) (b,e) or temozolomide(TMZ) (c,f) evaluated for 24, 48 and 72 h. Mean of three independent experiments ± standard error. *p<0.05, p<0.01, ***p<0.001

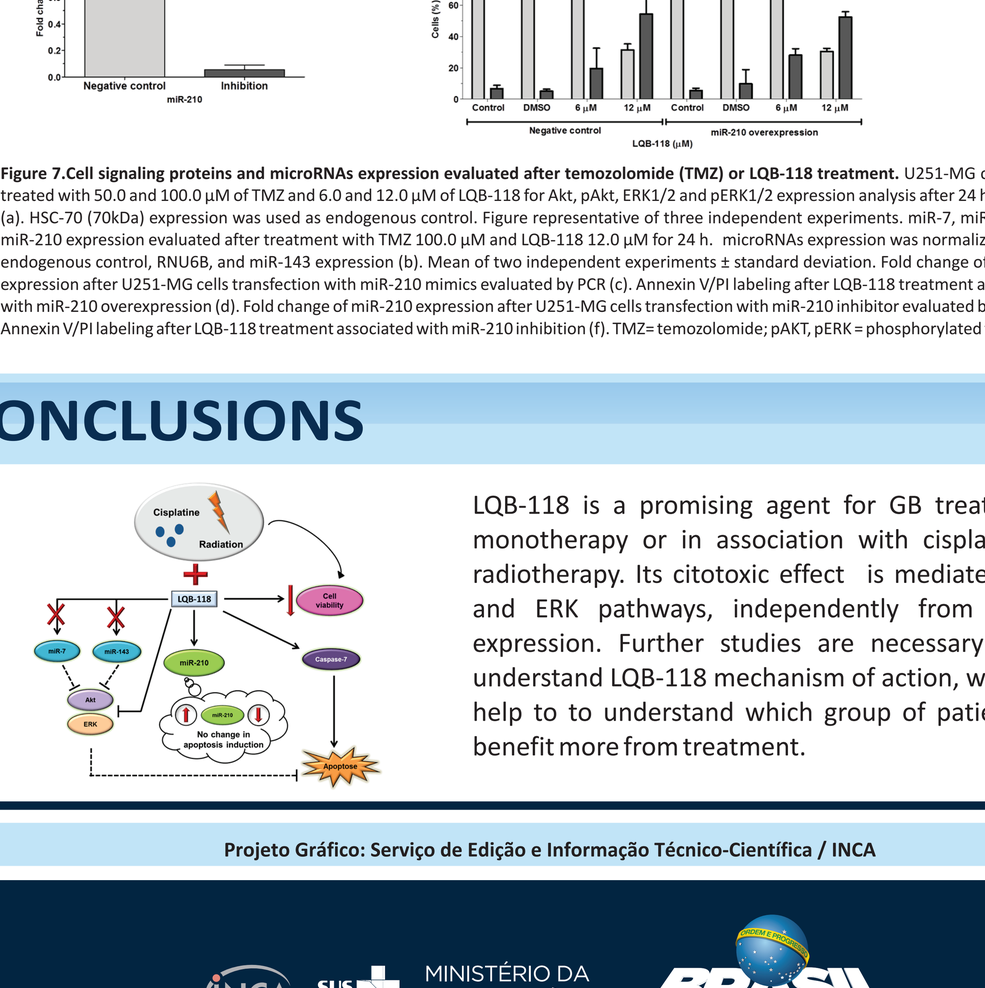

Figure 7.Cell signaling proteins and microRNAs expression evaluated after temozolomide (TMZ) or LQB-118 treatment. U251-MG cells were treated with 50.0 and 100.0 μM of TMZ and 6.0 and 12.0 μM of LQB-118 for Akt, pAkt, ERK1/2 and pERK1/2 expression analysis after 24 h and 48 h (a). HSC-70 (70kDa) expression was used as endogenous control. Figure representative of three independent experiments. miR-7, miR-143 and miR-210 expression evaluated after treatment with TMZ 100.0 μM and LQB-118 12.0 μM for 24 h. microRNAs expression was normalized by the endogenous control, RNU6B, and miR-143 expression (b). Mean of two independent experiments ± standard deviation. Fold change of miR-210 expression after U251-MG cells transfection with miR-210 mimics evaluated by PCR (c). Annexin V/PI labeling after LQB-118 treatment associated with miR-210 overexpression (d). Fold change of miR-210 expression after U251-MG cells transfection with miR-210 inhibitor evaluated by PCR (e). Annexin V/PI labeling after LQB-118 treatment associated with miR-210 inhibition (f). TMZ= temozolomide; pAKT, pERK = phosphorylated form

CONCLUSIONS

LQB-118 is a promising agent for GB treatment as monotherapy or in association with cisplatine and radiotherapy. Its citotoxic effect is mediated by Akt and ERK pathways, independently from miR-210 expression. Further studies are necessary to fully understand LQB-118 mechanism of action, which may help to to understand which group of patients may benefit more from treatment.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA