TG02, an oral CDK inhibitor, induces caspase-independent, non-autophagic cell death in human glioma cell lines and glioma-initiating cells

Emilie Le Rhun1,2, Caroline Von Achenbach1, Emese Szabo1 and Michael Weller3
(1) Department of Neurology, University Hospital and University of Zurich, Zurich, Switzerland, (2) University Hospital Lille, Lille, France

Background
TG02 is an orally bioavailable, multikinase inhibitor which potently inhibits cyclin-dependent kinases (CDK) 1, 2, 5, 7 and 9. CDK5-dependent depletion of short-lived oncoproteins such as MCL-1 and MYC has been proposed as its primary mechanism of cytotoxicity.

TG02 target gene expression is not related to outcome in glioblastoma: an analysis of the TCGA database

Kaplan-Meier survival curves of overall survival are shown for newly diagnosed glioblastoma patients. Patients were divided into two groups with high (blue) or low (red) expression of the direct kinase targets, CDK1, 2, 5, 7 or 9 (A), or the indirect targets MYC, MCL-1 and XIAP (B), based either on median mRNA expression level or expression level that results in the highest association with survival (lower nce). Statistical significances (p) were determined using the log-rank test (p<0.05 was considered significant).

TG02 target gene expression does not predict TG02 sensitivity

mRNA expression of direct targets (CDK 1, 2, 5, 7, 9) and indirect targets (MYC, MCL-1, XIAP) of TG02 was determined by qRT-PCR. No specific cell cycle alteration, but increased Annexin labeling suggestive of apoptosis in response to TG02

Protein levels of direct targets (CDK 1, 2, 5, 7, 9) and indirect targets (MYC, MCL-1, XIAP) were assessed by immunoblot.

Minor caspase 3 processing and a protective role of autophagy?

Cells treated accordingly were assessed for caspase 3 or LC3 protein patterns by immunoblot. Parallel cultures were pretreated (1 h) and cotreated with TG02 for 72 h. 3-Methyladenine treatment enhanced growth inhibition mediated by TG02, suggesting that autophagy is protective.

Hypoxia confers resistance to TG02 to GIC cultures, but not to LTC cultures

Under hypoxic culture conditions, TG02 was less active against the glioma-initiating cell lines, LN-161 and ZH-305, but not against the long-term cell lines, LN-229 and LN-308.

Conclusions
Baseline expression of direct TG02 targets (CDK-9, CDK-5) and indirect TG02 targets (MCL-1, MYC) is detected in all cell lines by immunoblot, but does not correlate with sensitivity to TG02. TG02 exhibits strong anti-tumor activity in both human long-term cell lines (LN-18, LN-428, D247MG, LN-319, A172, U87MG, T98G, LN-308, LN-229) and glioma-initiating cell lines (T325, T269, ZH-161, ZH-305) in vitro regardless of MGMT promoter methylation status (not shown).

TG02 is a highly potent anti-glioma agent in vitro with a novel mode of action that induces cell death in a largely caspase-independent, non-autophagic manner. Further cell death studies are ongoing. Early clinical trials of TG02 in glioblastoma are ongoing.