ONC201/TIC10 has a strong anti-neoplastic activity in medulloblastoma cells independent of c-Myc and is synergistically enhanced by 2-DG

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Background

Medulloblastoma represents one of the most common brain tumors in children. While the understanding of the molecular characteristics of this disease has very much advanced, more efficient and less toxic therapeutics are still in high demand. In this study we examined whether the imipridone ONC201/TIC10 affects the metabolic and proliferative activity of medulloblastoma cells alone and in combination with 2-Deoxyglucose in vitro.

Methods

Extracellular flux (agilent seahorse) and Western blot analyses were performed to assess effects on tumor cell metabolism and the expression of proteins of the respiratory chain in established medulloblastoma cells. MTT assays and spheroid assays were performed to examine anti-proliferative effects in a 2-D and 3-D setting.

Results

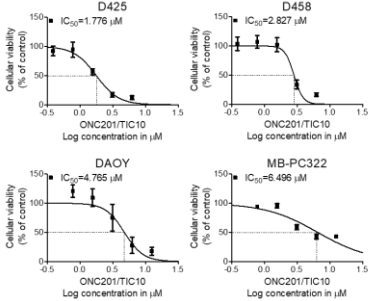

Figure 1: Dose response curves of ONC201/TIC10 in indicated medulloblastoma cells after 72h of treatment. Non-linear regression was performed and IC50-values were calculated.

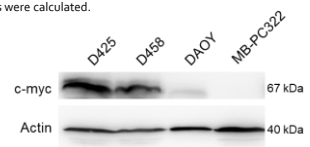

Figure 2: Western blots showing basal c-Myc expression in indicated medulloblastoma cells.

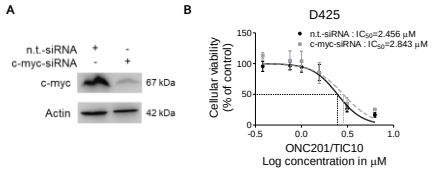

Figure 3: D425 cells were treated with n.t.-siRNA or c-Myc siRNA. A, Western blots confirming down regulation of c-Myc expression following treatment with c-Myc-siRNA. B, Dose response curves of ONC201/TIC10 after 72h of treatment in D425 cells treated before either with n.t.-siRNA or c-Myc-siRNA.

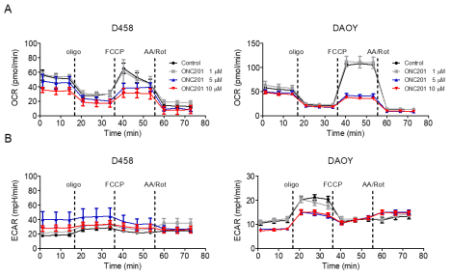

Figure 4: Extracellular flux analysis was performed in D458 and DAOY cells following treatment with increasing concentrations of ONC201/TIC10. Oxygen consumption rate (OCR) graphs (A) and extracellular acidification rate (ECAR) graphs (B) of a mitochondrial stress test.

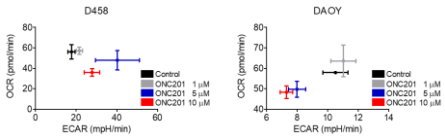

Figure 5: OCR/ECAR graphs of D458 and DAOY cells treated with increasing concentrations of ONC201/TIC10 under basal condition.

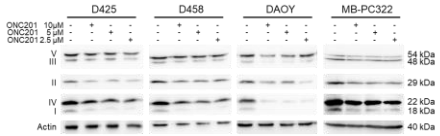

Figure 6: Indicated medulloblastoma cells were treated for 24h with increasing concentrations of ONC201/TIC10. Western blots were performed to determine the expression of respiratory chain enzymes (complexes I-V).

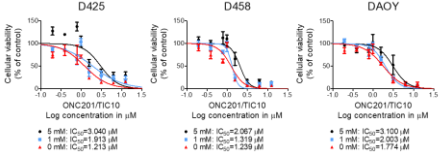

Figure 7: Glucose starvation sensitizes for ONC201/TIC10. Medulloblastoma cells were treated with increasing concentrations of ONC201/TIC10 at indicated glucose levels for 72h. The cellular viability was assessed and IC50-values were calculated.

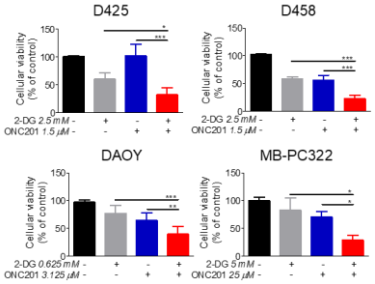

Figure 8: 2-Deoxyglucose enhances the anti-proliferative effect of ONC201/TIC10.

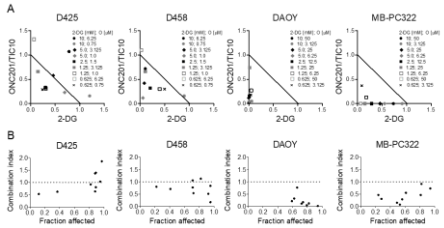

Figure 9: Different medulloblastoma cells were treated for 72h with indicated concentrations of ONC201/TIC10 and 2-DG prior to performing MTT assays. A, Isobolograms were calculated to determine the characteristics of the drug-drug interaction. B, Combination index/FA graphs for cells treated as described for A. CI<1: synergism, CI=1: additivity, CI>1: antagonism.

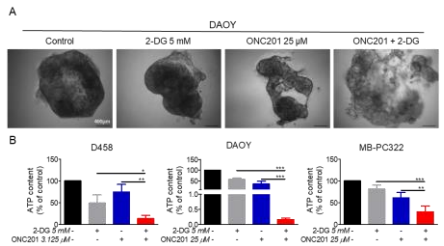

Figure 10: Combined treatment with ONC201/TIC10 and 2-DG yields synergistic inhibition of the growth of spheroids. Spheroids were allowed to form for one week prior to treatment during one more week. A, Representative microphotographs of DAOY spheroids treated as indicated. B, Quantitative representation of spheroids treated as described for A.

Conclusion

Overall, ONC201/TIC10 profoundly inhibits the proliferative activity of medulloblastoma cells in a c-Myc-independent manner. Additional treatment with the glycolysis inhibitor 2-Deoxyglucose synergistically enhances the anti-medulloblastoma activity of ONC201/TIC10. Further investigations are warranted.