



**HKU
Med**

LKS Faculty of Medicine
The University of Hong Kong
香港大學李嘉誠醫學院

Vitamin D Enhances Temozolomide Anti-Tumour Efficacy in Human Glioblastoma Multiforme: *In Vitro* and *In Vivo* Studies

LO Sze Ching, KIANG Mei Yee, LEUNG Gilberto Ka Kit

Division of Neurosurgery, Department of Surgery

The University of Hong Kong

Background

- Grade IV glioma, known as glioblastoma multiforme (GBM), is among the most malignant primary brain tumours. Its high proliferation and common resistance to conventional therapies complicate treatment, making it lethal in many cases. The hormonally active form of vitamin D (VD), calcitriol (1 α ,25(OH)2D3), regulates many cellular physiological processes including cell proliferation, differentiation and apoptosis. [1]
- Supraphysiological doses of VD have shown anti-tumour efficacy in the pancreas, liver, breast and prostate. [2] There is however a lack of studies in human GBM in terms of its combination treatment effect and the mechanism by which it brings about anti-tumour efficacy.
- This study aims to characterize the anti-tumour efficacy of VD supplementation in human GBM to determine whether combination with temozolomide (TMZ) can improve disease outcome, its mechanism of action, and a potential application on VD-deficient subjects.

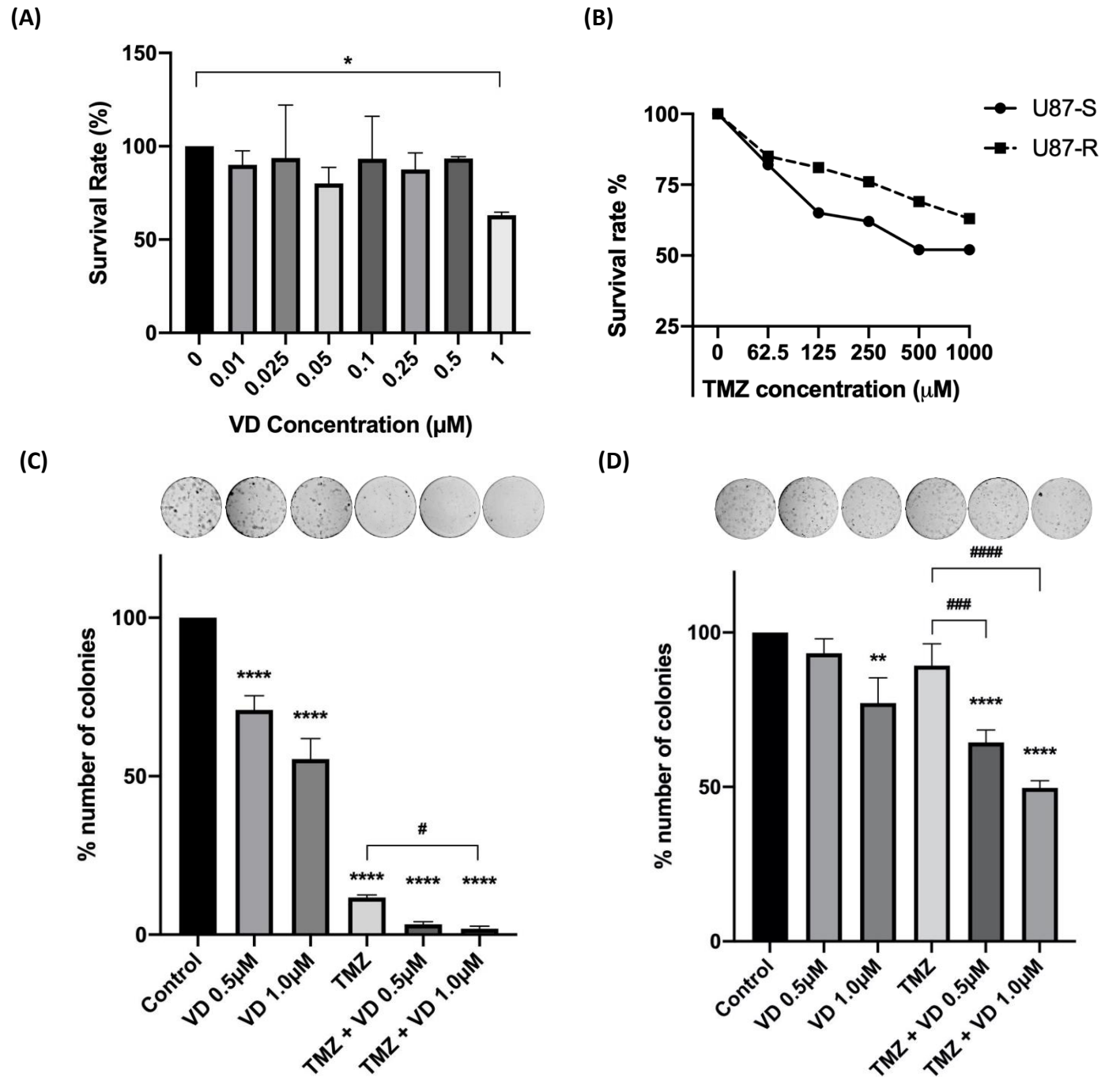
Methodology

- *In vitro* studies:
 - MTT assays were performed to determine cytotoxicity and the half-maximal inhibitory concentration (IC_{50} value) of VD on human U87 GBM cells
 - Clonogenic assays were performed on human U87 GBM cells, using both sensitive (S) and resistant (R) cell lines, to determine the effect of VD, in combination with and without TMZ, on GBM cell survival
- *In vivo* studies:
 - A mice subcutaneous xenograft model was used. U87S cells were implanted subcutaneously in immunocompromised nude mice, and the mice were divided into 4 groups (n=3 animals per group). The first group was vehicle control (injected intraperitoneally with 100 μ l oil), the second group was treated with TMZ (55mg/kg/day by oral gavage) dissolved in 100 μ l saline, the third group was injected intraperitoneally with 0.2 μ g/kg/day VD dissolved in 100 μ l oil, and the fourth group received both the TMZ and VD as described above. The treatment duration was 12 days, with TMZ given for 3 consecutive days/week, and VD given every day. Tumour size was measured every three days for 12 days.

Results

- MTT assays showed the optimal IC₅₀ dose of VD on U87 cells to be 1 μM
- Clonogenic assays showed that VD reduces U87 cell survival when combined with TMZ in both U87S and U87R cell lines, with optimal inhibition shown when combined with 0.1 μM VD

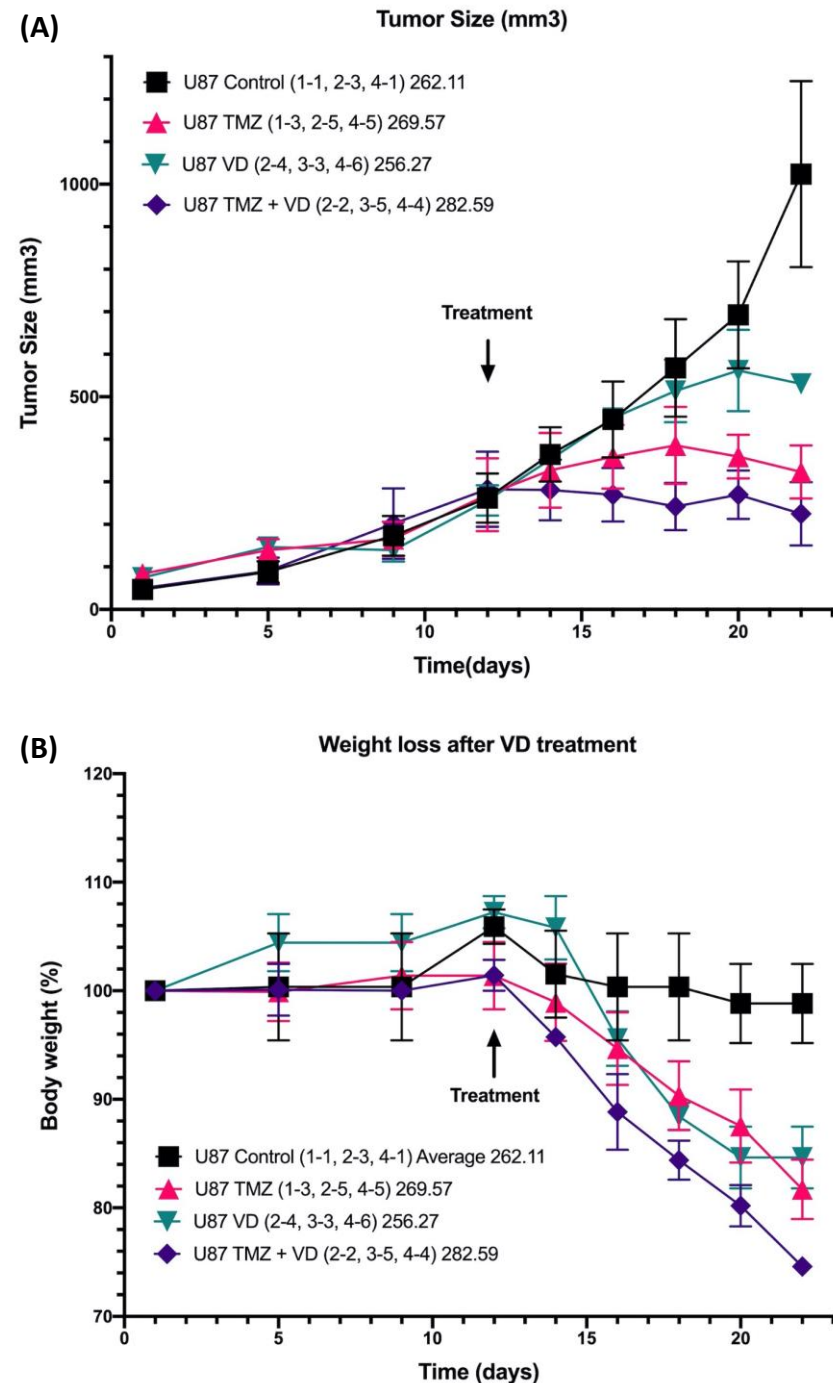
Fig 1. *In vitro* effects of VD on U87 cell lines. (A) Graph of VD concentration (μM) against U87S survival rate (%) in MTT assay. (B) Graph of TMZ concentration (μM) against U87S/R cell lines survival rate (%) in MTT assay. (C) Graph of VD ± TMZ on U87S cell line in clonogenic assay. (D) Graph of VD ± TMZ on U87R cell line in clonogenic assay. *P<0.05, **P<0.01, ***/####P<0.001, ****/#####P<0.0001. VD, vitamin D; TMZ, temozolomide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.



Results

- The subcutaneous tumour xenograft model showed a greatest reduction in tumour growth when treated with TMZ and VD, compared to TMZ alone and VD alone. This indicates that TMZ and VD combined exhibit a synergistic anti-tumour effect on the growth of human U87S GBM cells

Fig 2. *In vivo* effects of VD on U87 cell lines. (A) Graph of U87S tumour size against treatment time, each treated with oil (vehicle), VD, TMZ or TMZ + VD. (B) Graph of weight loss (%) against treatment time. VD, vitamin D; TMZ, temozolomide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.



Discussions and Conclusion

- Current findings validated the role of VD in reducing human GBM proliferation and inducing apoptosis, and lay the groundwork for further *in vivo* investigations on the mechanism of action of the synergistic effect of VD combined with TMZ
- The investigation on the role of VD supplementation against human GBM also raises interest in its effect in a VD-deficient environment, and may serve as a guide towards treating GBM in VD-deficient patients and hence deserve further investigation

References:

1. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev.* 2016;96(1):365-408. doi:10.1152/physrev.00014.2015
2. Campbell MJ, Trump DL. Vitamin D Receptor Signaling and Cancer. *Endocrinol Metab Clin North Am.* 2017;46(4):1009-1038. doi:10.1016/j.ecl.2017.07.007