A pilot evaluation of the dose- and YAP-dependent effects of verteporfin in canine and mouse osteosarcoma cells.

Edvin Pepeljak[1&], Ana Valeria Vilchis Celis[1,2,&], Aaron Dhillon[1], Jeff Hummel[3], Alicia Viloria-Petit[1]*

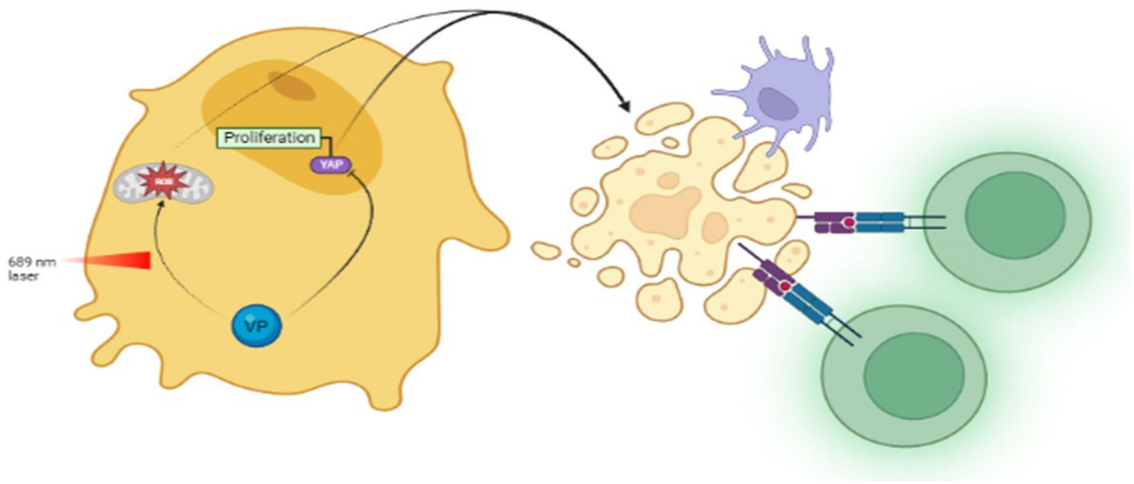
[1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Department of Morphology, National Polytechnic Institute, Ciudad de Mexico, CDMX, Mexico, [3]Illumacell Inc, Hamilton, ON, Canada,
[&]Authors contributed equally, *Principal Investigator.

Introduction

- Osteosarcoma (OS) is the most common bone cancer in dogs, and the majority of patients will succumb to metastatic disease.
- Photodynamic therapy (PDT) using verteporfin (VP) shows promise for canine OS treatment and inhibits tumor growth both with and without light activation by targeting YAP and inducing immunogenic cell death

Objective

To demonstrate that VP will cause ICD of OS cells in a YAP-targeting and dose-dependent manner, with maximum effects following photo-activation.



Methods and Materials

Cell Culture:

- metastasis-derived canine (D17 and OVC-cOSA-31) and mouse (K7M2) OS cell lines were cultured following standard protocol with DMEM + 10% FBS and antibiotics

Verteporfin and YAP:

- Cells were treated with increasing doses of VP for 24 hours without light exposure.
- The effects of VP treatment on YAP levels were evaluated through immunoblotting

YAP-Targeting and Minimally-Targeting Dose Treatment:

- Cells were then divided into two treatment groups: YAP-targeting dose and minimally-targeting dose of VP.
- Within each treatment group, cells were further divided into two subgroups: with and without activating light (LED, 635 nm).

Cell Viability, Apoptosis, and Immunogenic Cell Death (ICD) Assessment:

- Cell viability was determined using a WST-1 assay
- Apoptosis was analyzed through immunoblotting using specific apoptotic markers
- ICD induction was evaluated using immunofluorescence staining.

Results

VP downregulates YAP expression in a dose-dependent manner.

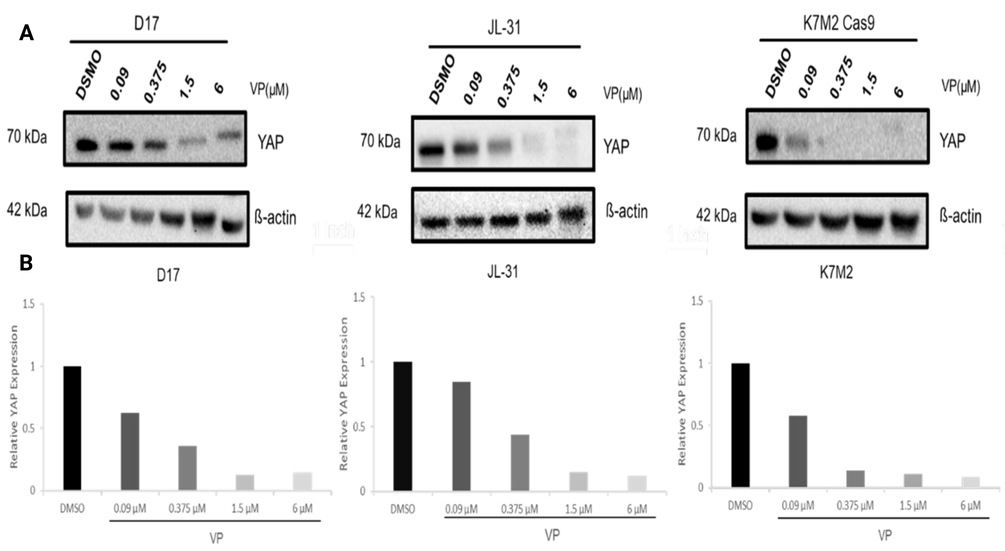


Figure 1. (A) Representative Western blot showing decreased YAP levels with higher doses of VP, with β-actin used as a loading control. (B) Densitometry analysis used for quantifying YAP expression, with protein levels normalized to β-actin and control to determine relative expression.

YAP targeting and minimal YAP-reducing VP doses induce Apoptosis

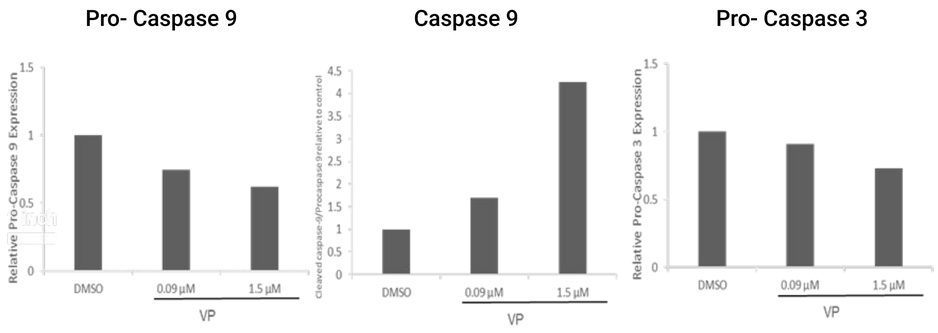


Figure 2. Representative Western blot of JL-31 cells illustrating reduced levels of pro-caspase 3 and 9, accompanied by an increase in the relative expression of cleaved caspase 9 following treatment with both YAP-targeting and minimal YAP-reducing doses of VP. This pattern is indicative of induction of apoptosis.

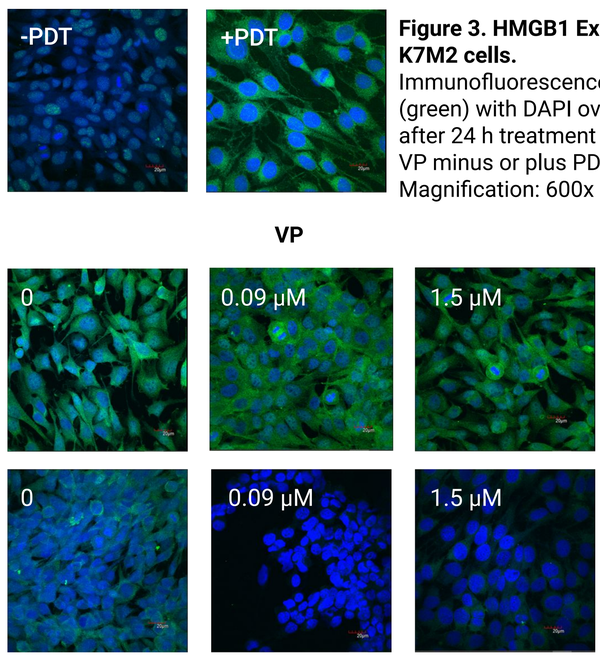


Figure 3. HMGB1 Expression in K7M2 cells.
Immunofluorescence of HMGB1 (green) with DAPI overlay (blue) after 24 h treatment with 1.5μM VP minus or plus PDT. Magnification: 600x

Figure 4. YAP expression in K7M2 cells.
Immunofluorescence images of YAP (green) with DAPI overlay (blue) without and with PDT treatment, in the absence (control) or presence of VP at the indicated concentrations. Magnification: 600x

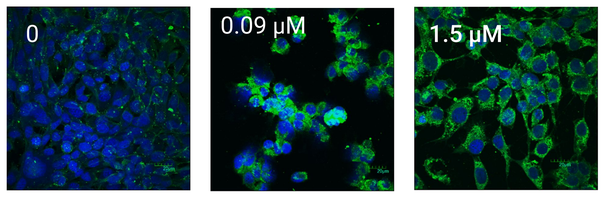


Figure 5. HSP90B1 expression in K7M2 cells.
Immunofluorescence of HSP90B1 (green) with DAPI overlay (blue) after PDT treatment with VP at the indicated concentrations. Magnification: 600x

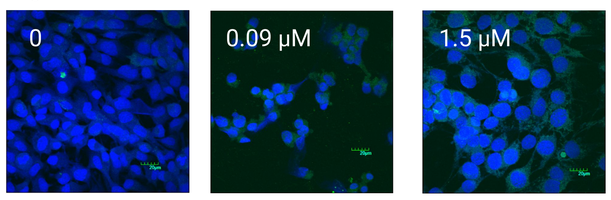


Figure 6. CC3 expression in K7M2 cells.
Immunofluorescence of CC3 (green) with DAPI overlay (blue) after PDT treatment with VP at the indicated concentrations.
Magnification: 600x

Conclusions

- YAP-targeting doses of VP were more effective in reducing cell viability when combined with activating light
- YAP-targeting doses induced higher rates of apoptosis
- Changes in HMGB1 localization and increase in HSP90B1 expression in K7M2 cells after treatment with VP PDT suggests ICD
- Expression of CC3 after VP PDT treatment indicates apoptosis
- Changes in YAP location and YAP expression following VP treatment indicates possible interactions between VP and YAP

Overall, our results indicate that a YAP-targeting dose of VP is more effective at reducing viability of canine OS cells and at promoting ICD when activated by light.

Acknowledgements

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