Supplementary Online Content

Wu J, Wood GS. Analysis of the Effect of Gentian Violet on Apoptosis and Proliferation in Cutaneous T-Cell Lymphoma in an In Vitro Study. *JAMA Dermatol.* Published online August 29, 2018. doi:10.1001/jamadermatol.2018.2756

This supplementary material has been provided by the authors to give readers additional information about their work.

Supplemental Figures

Supplemental Figure 1. Gentian Violet induced apoptosis involves extrinsic and intrinsic pathways.

Cells were treated with gentian violet (+) or DMSO (-) at 1uM for 24 hours and cleaved caspases 8, 9, 3 and MCL1 were detected by Western Blot. M: MyLa, Se: SeAx.

SUPPLEMENTAL FIGURE 1- IMMUNOBLOTS CONFIRM THE INCREASES IN THE APOPTOTIC FACTORS NOTED IN FIGURE 2 AS WELL AS REDUCTIONS IN ANTI-APOPTOSIS FACTOR MCL1.

Supplemental Figure 2. Levels of TNF pathway factors are not altered by gentian violet

Cells were treated with gentian violet, nitrogen mustard or DMSO at 1uM for 24 hours. TNF pathway proteins were detected by flow cytometry. Y-axis shows MFI fold change. White column: DMSO; black: gentian violet; gray: nitrogen mustard. No significant differences were detected.

SUPPLEMENTAL FIGURE 2 – GENTIAN VIOLET DOES NOT AFFECT TNFa LIGAND OR TNF-R1 DEATH RECEPTOR IN CTCL TUMOR CELLS – BAR GRAPHS SHOW STABLE LEVELS OF THESE PROTEINS.

Supplemental Figure 3. Gentian Violet leads to up-regulation of phosphorylated phospholipase C-gamma 1 (pPLC-gamma 1), calcium influx and reactive oxygen species (ROS).

Cells were treated with gentian violet, nitrogen mustard or DMSO at 1uM for 24 hours. Key factors upstream of FASL production were detected by flow cytometry. Y-axis shows MFI fold change. White column: DMSO; black: gentian violet; gray: nitrogen mustard. **: $P<0.01$ compared to DMSO, ++: $P<0.01$ compared to nitrogen mustard.

SUPPLEMENTAL FIGURE 3 – GENTIAN VIOLET INCREASES LEVELS OF PHOSPHORYLATED PHOSPHOLIPASE C-GAMMA 1 (pPLC-GAMMA 1), CALCIUM INFLUX

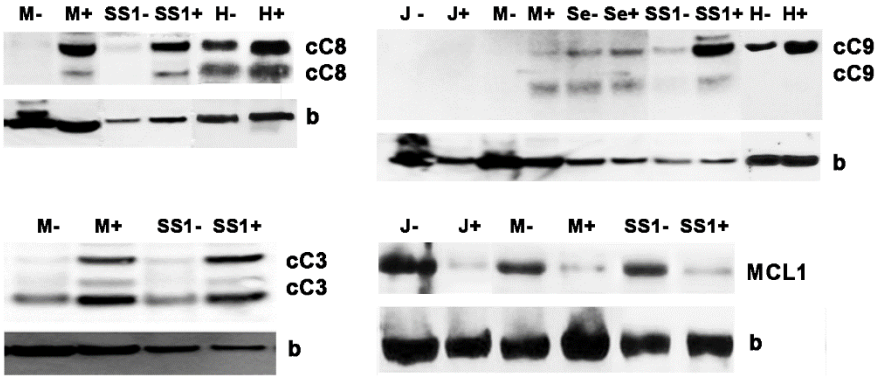
AND REACTIVE OXYGEN SPECIES (ROS) IN CTCL TUMOR CELLS – BAR GRAPHS SHOW INCREASED LEVELS OF pPLC-GAMMA 1, CALCIUM INFLUX AND ROS.

Supplemental Figure 4. Gentian Violet leads to decreased NF-kB subunit expression and increased IkB.

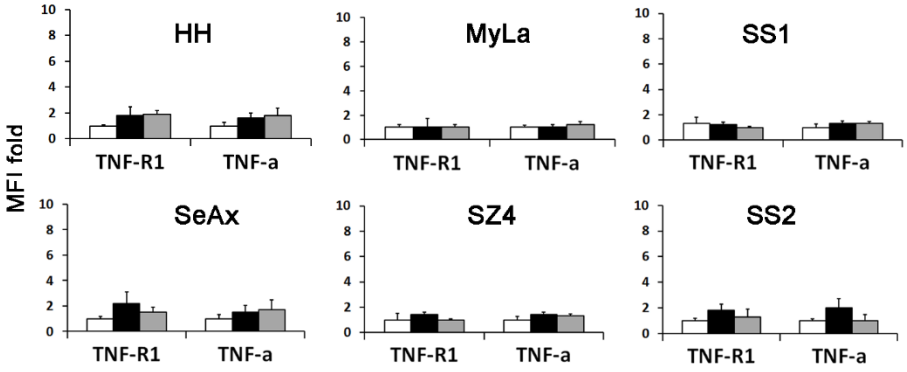
Cells were treated with gentian violet (+) or DMSO (-) at 1uM for 24 hours. NF-kB subunits and IkB were detected by Western Blot. M: MyLa.

SUPPLEMENTAL FIGURE 4 – GENTIAN VIOLET INHIBITS NFkB PROLIFERATIVE DRIVERS IN CTCL TUMOR CELLS – IMMUNOBLOTS SHOW DECREASED LEVELS OF NFkB SUBUNITS AND INCREASED NFkB INHIBITOR IkB.

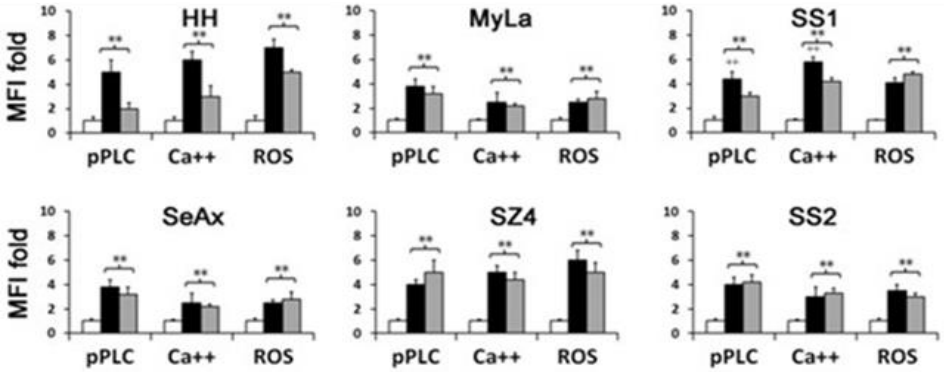
Supplemental figure 1



Supplemental figure 2



Supplemental figure 3



Supplemental figure 4