Characterizing Sensitivity to Vincristine, Irinotecan, and Telomerase-targeted Therapy in Diffuse Anaplastic Wilms Tumor Patient-derived Xenografts

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Background

Overall survival rates for children with Wilms tumor (WT) are above 90%, but those with advanced-stage diffuse anaplastic WT (DAWT) and those with favorable histology (FHWT) disease relapse suffer relatively poor outcomes. An ongoing clinical trial is studying the effect of addition of vincristine and irinotecan (VI) to an established chemotherapeutic regimen on survival outcomes in patients with newly-diagnosed DAWT and standard-risk relapsed FHWT patients. However, there is limited published data on responsiveness to VI in WT cell lines and patient-derived xenografts (WTPDX). Our group previously described that telomerase activity is upregulated by various mechanisms in anaplastic WT. We sought to characterize the *in vivo* and *in vitro* sensitivity of anaplastic WTPDX and WT cell lines to vincristine and irinotecan with or without the telomerase-targeted drug 6-thio-2'-deoxyguanosine (6dG) to assess for synergistic response.

Methods

BALB/c scid mice bearing anaplastic histology heterotopic WTPDX (KT-51, KT-53, KT-60), were treated with 0.5 mg/kg vincristine (Q7DX3) IP and 0.625 mg/kg irinotecan (DX5)2 IP, or 0.5 mg/kg 6dG (DX5)3 IP, or in combination. Tumor volumes were measured weekly up to 12 weeks. WTPDX-derived tumor spheroids were generated. Drug sensitivities to vincristine, SN-38 (active metabolite of irinotecan), and 6dG were measured in adherent anaplastic WT cell lines (PDM115, WiT49) and in WTPDX-derived spheroids, (KT-51, KT-53, KT-71). Colony formation assays were performed on cell lines using crystal violet staining. DNA damage response to 6dG monotherapy was assessed in 2D culture with WiT49 and in tumor spheroids with KT-51 and KT-53.

Results

A median complete response was noted *in vivo* to VI in KT-53 and KT-60 and partial delay in tumor growth in KT-51. A partial delay to no response was found with 6dG monotherapy in KT-51, KT-53, and KT-60 with no additive suppression with VI combination. *In vitro* studies demonstrated variable sensitivity to vincristine (IC50 0.008-14.8 nM) and were more similar in their sensitivity to irinotecan (2.4-61.3 nM), with all being resistant to 6dG monotherapy (all 5+ uM). In 2D culture, 6dG + VI exhibited minimal synergistic effect in reducing colony formation in WiT49 cells. 6dG monotherapy induced variable DNA damage response in KT-51, KT-53, and WiT49.

Conclusion

Anaplastic WTPDX exhibit sensitivity in vivo and in vitro to vincristine and irinotecan but are resistant to telomerase-targeted 6dG monotherapy or in combination with VI. Future research will determine mechanisms of resistance to VI and identify targeted therapies that exhibit synergy with VI in WTPDX models.

One-Liner: Anaplastic Wilms tumor xenografts exhibit sensitivity to vincristine and irinotecan (VI) combination therapy but appear to be resistant to telomerase-targeted therapy with 6-deoxy-2'-thioguanosine (6dG) monotherapy or in combination with VI.

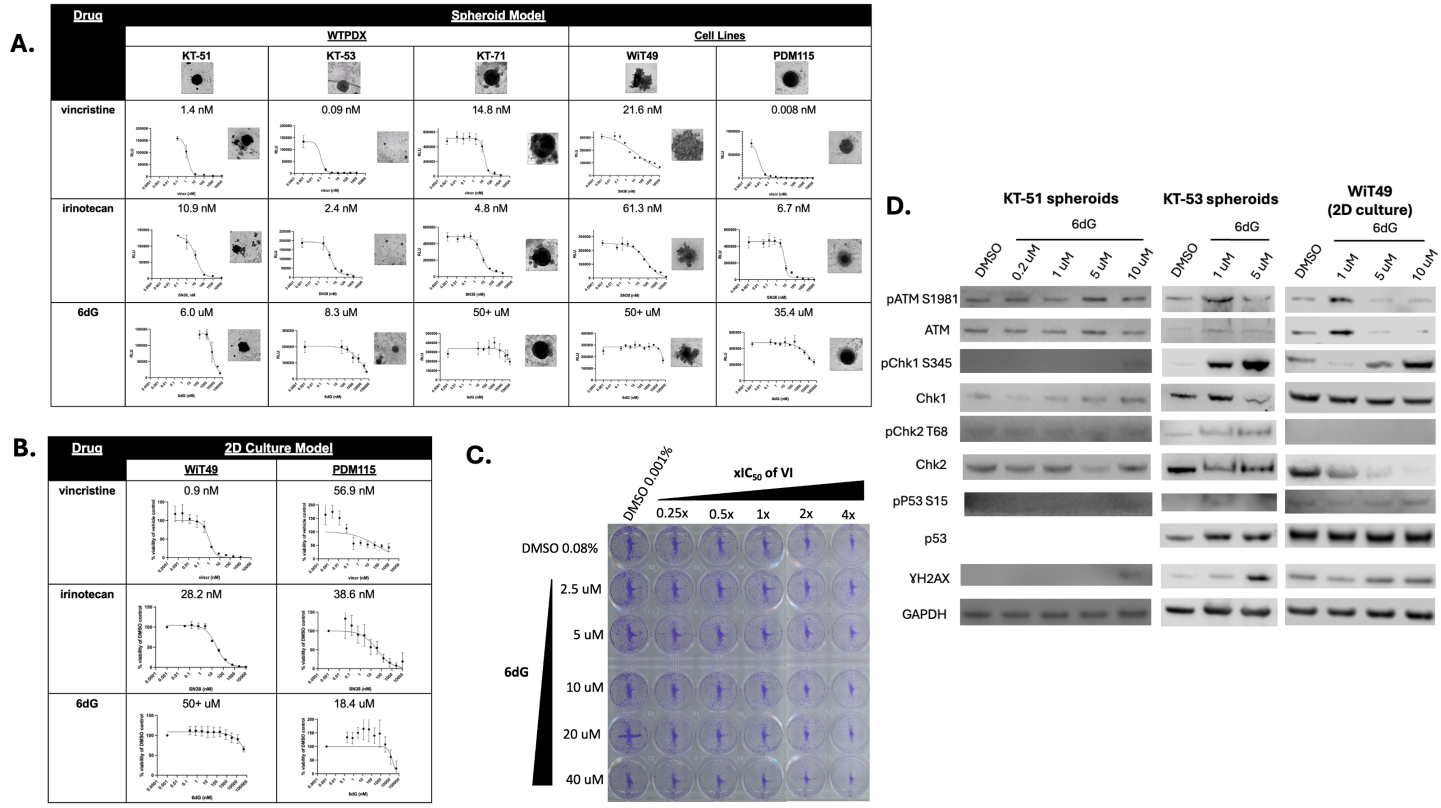

Figure. *In vitro* sensitivity to vincristine, irinotecan, and 6dG in anaplastic WTPDX (3D culture spheroids) and WT cell lines (2D adherent and 3D spheroids). A: Spheroids were generated via collagenase digestion of harvested tumors, plating and centrifuging 5-10k viable cells in ultra-low attachment round-bottom 96-well plates and treating after culturing 7d (WTPDX) or 2d (cell lines) with variable doses of drug for 5-7 days. Spheroid viability was assessed using CellTiterGlo3D. IC50s were calculated with nonlinear regression using Graphpad. Representative images of spheroids at end of treatment period for vehicle control (top column) or high doses of 6dG (50 uM) or vincristine/irinotecan (500 – 1000 nM) are included; images scaled to 1mm^2. B: Adherent anaplastic WT cell lines WiT49 and PDM115 were plated on opaque flat 96-well plates at 3000 cells/well and treated with varying doses of drug 24h post-plating for 5d total. Viability was assessed with PrestoBlue and IC50s were calculated with Graphpad. C: Colony formation assay of VI and 6dG in WiT49; 10,000 cells/well were seeded on 12-well plates and treated with combinations of 6dG and vincristine/irinotecan after 24h. Plates were stained with crystal violet after 1 week of treatment. D: Western blots for DNA damage response markers in lysates of KT-51 and KT-53 spheroids and WiT49 2D culture after treatment with 6dG (5d in spheroids, 3d in 2D culture).