WNC0901 is a novel DNA-PK inhibitor and potent radiosensitizer of otherwise radioresistant solid tumors

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ABSTRACT

BACKGROUND

Therapeutic resistance limits the efficacy of radiation therapy in many tumors and provides a compelling rationale to develop novel treatments to enhance radiation sensitivity. DNA-dependent protein kinase (DNA-PK) is a major mediator of DNA repair following radiation, and DNA-PK inhibitors are effective radiosensitizers. WNC0901, developed by Waynola Biopharma, is a novel DNA-PK inhibitor. The focus of this project is to evaluate the radiosensitizing effects of WNC0901 radioresistant histologies.

RESULTS

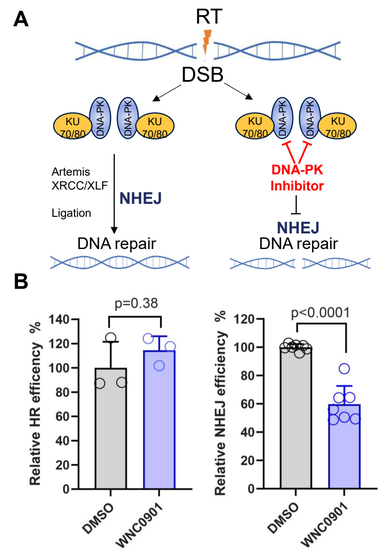
WNC0901 is a potent DNA-PK inhibitor in both a cell-free kinase assay (IC50 = 0.073 nM) and a cell-based assay (IC50 = 76 nM). WNC0901 has a 30-fold specificity for DNA-PK relative to other PI3K-related kinases. In a broader kinome screen (363 kinases), 1 mM WNC0901 resulted in >25% inhibition of only five other kinases. There is robust inhibition of radiation-induced DNA-PK autophosphorylation (Ser-2056), as analyzed by Western blotting, with maximal effect at 300 nM WNC0901 in A549 (lung adenocarcinoma) and U2OS (osteosarcoma). The efficacy of WNC0901 as a radiosensitizer was evaluated in clonogenic assays across these same two radioresistant tumor models. In a full radiation clonogenic, the sensitizer enhancement ratio at 10% survival (SER10) was 3.8 (A549) and 2.3 (U2OS). In vitro metabolism studies have identified demethylated WNC0901 (WNC0901-M) as a major metabolite. Similar to the parent drug, WNC0901-M is a potent DNA-PK inhibitor with maximal radiosensitizing effects of the metabolite observed at 300nM with SER10 of 2.4 (A549) and 1.9 (U2OS).

The in vivo efficacy of 50 mg/kg WNC0901 combined with 8 Gy was evaluated and compared to another DNA-PK inhibitor, 50 mg/kg peposertib, in A549 tumor heterotopic xenografts. Compared to placebo (median time to endpoint 63 days) or RT alone (median 72 days), the combination of WNC0901 and RT significantly delayed tumor regrowth (median 169 days; p=0.002 compared to RT). Interestingly, peposertib + RT was ineffective (median 83 days; p=0.332 compared to RT). In a second study, similarly robust sensitizing effects of WNC0901 was observed in HT29 (colorectal) xenografts (placebo - median 45 days; RT – median 75 days; RT + WNC0901 – median >110 days; p=.03 relative to RT).

CONCLUSIONS

Together, these findings demonstrate the robust radiosensitizing effects of WNC0901 in radioresistant tumors and provide a rationale for continued drug development.

DNA-PK SIGNALING

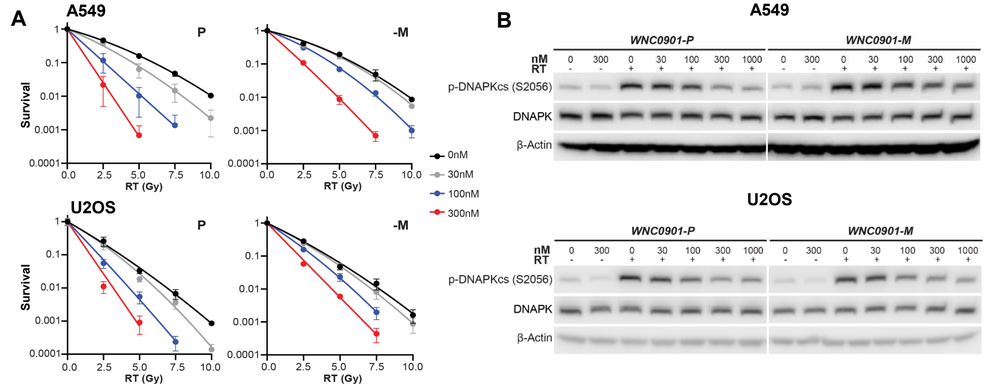


A. DNA-PK inhibitor mechanism of action[1,2]. DSB: DNA double-strand breaks; NHEJ: non-homologous end joining.
B. Reporter assay in A549 (lung) cells treated with 300nM WNC0901.

WNC0901 PROPERTIES

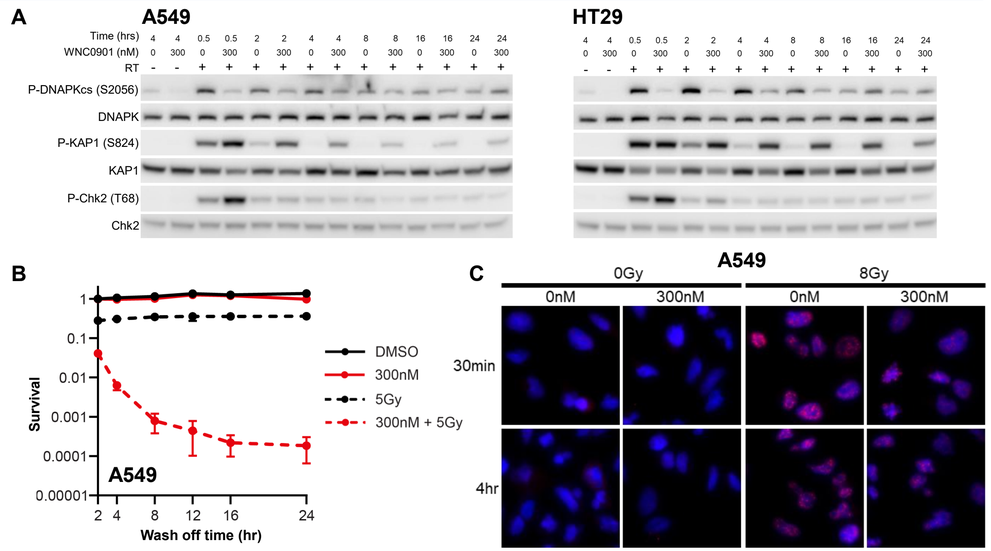
Studies	WNC0901
DNA-PK IC50, cell-free (nM)	0.073
pDNA-PK inhibition after 10Gy in HT-29 cells (nM)	75.5
ATM/ATR/PI3K/mTOR IC50	>30-fold
Human, Rat plasma unbound (% free)	67.5%/52.6%
Rat brain unbound (% free)	58.40%
Rat Kp,uu	0.026
Human, Rat hepatocyte CLint (ul/min/10^6 cells)	<1/2.86
Aldehyde Oxidase stability in Human liver cytosol; Clint(ul/min/mg)	0.44 $T^1/_2$ = 2392 min
Papp x 10^{-5}cm/s	12.5
ER P-gp/BCRP	3.1/3.1
In vivo $t_{1/2}$ / Rat oral bioavailability	6.2h/70.8%
In vivo Rat clearance (ml/min/kg)	20.5

RADIOSENSITIZING EFFECTS



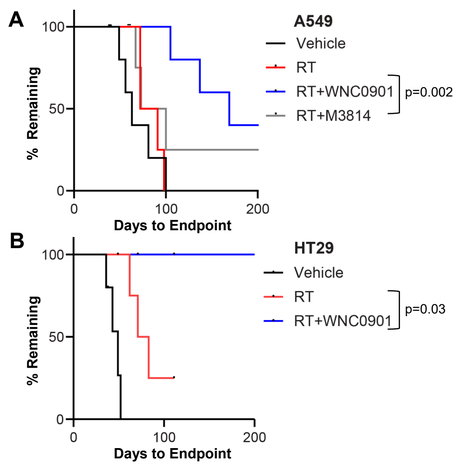
A. Clonogenic results following 14-day incubation after treatment with WNC0901 (P) and demethylated WNC0901 (WNC0901-M) +/- radiation (RT) in A549 (lung) and U2OS (osteosarcoma) cell lines. **B.** Western blot data. Lysates harvested 30m following radiation (RT) with WNC0901 (P), WNC0901-M, +/- single 10 Gy dose.

TIME COURSE ANALYSIS



A. Western blot performed in A549 (lung) and HT29 (colorectal) cell lines. Cells harvested at the timepoints indicated post treatment with 300nM of WNC0901 +/- 10Gy RT. **B.** Clonogenic results following 14day incubation after drug wash off at the timepoints indicated. **C.** A549 pDNA-PK immunofluorescence at 30min and 4hr fixation after RT.

IN VIVO FLANK EFFICACY



A. A549 flank model treated with single fraction 8 Gy RT +/- 50 mg/kg WNC0901 or 50mg/kg M3814 and followed for overall time to endpoint. **B.** HT29 flank model treated with single fraction 8 Gy RT +/- 50mg/kg WNC0901 and followed for overall time to endpoint.

FUTURE DIRECTIONS

1. Evaluate the effects of WNC0901 in gastrointestinal, skin, and central nervous system tissues using immunofluorescence.
2. Focus on biomarker discovery to identify a patient population that will benefit most from this treatment.
3. Optimize treatment parameters for potential use in patients.

REFERENCES, FUNDING, CONTACT

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2. Suwen Hu et al. (2021) Small molecule DNA-PK inhibitors as potential cancer therapy: a patent review (2010–present), Expert Opinion on Therapeutic Patents, 31:5, 435-452, DOI: 10.1080/13543776.2021.1866540

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