Preclinical data of a novel DNA-PK inhibitor in combination with radiation therapy shows promise in the treatment of established GBM and lung carcinoma cell lines

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Ann C. Mladek[1], Danielle M. Burgenske[1], William F. Elmquist[2], Wei Zhong[3], and Jann N. Sarkaria[14]

[1]Department of Radiation Oncology, Mayo Clinic, Rochester, MN, USA. [2]Department of Pharmaceutics- College of Pharmacy, University of MN, Minneapolis, Minnesota, USA. [3]Waynola Biopharm, Inc., Corona, CA, USA.

ABSTRACT

BACKGROUND

Radiation therapy (RT) is a cornerstone of oncologic therapy for the majority of solid malignancies, and unrepaired DNA double-strand breaks are responsible for radiation-induced cytotoxicity. RT-induced DNA lesions can be repaired by multiple mechanisms including homologous recombination (HR) and non-homologous end joining (NHEJ), and disruption of either of these pathways can enhance cytotoxicity. A key component of the NHEJ pathway is the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), and loss of kinase activity can significantly increase radiosensitivity.

OBJECTIVE

Herein we describe the results of our medicinal chemistry campaign to develop a potent and selective DNA-PKcs inhibitor, WNC0901.

RESULTS

WNC0901 inhibits DNA-PKcs kinase activity in a cell free system with an IC_{50} of 0.071 nM and demonstrated at least 30-fold higher sensitivity than other family members (ATM, ATR, mTOR, PI3K). WNC0901 has limited aldehyde oxidase (AO) liability with a $T_{1/2}$ >2000 min in liver cytosol and is stable for over 120 minutes in the presence and absence of an AO inhibitor. A preliminary pharmacokinetic analysis in wistar han rat (IV=2mg/kg, PO=20mg/kg) demonstrated 116% apparent absolute oral bioavailability, 2.6 hour terminal half-life, 33.0 mL/min/kg clearance, and a 2.6% unbound brain-to-plasma partition coefficient (Kpuu). WNC0901 also had favorable pharmacokinetic properties in beagle dog (IV=2mg/kg, PO=20mg/kg) with moderate clearance (8.5 mL/min/kg), high apparent absolute oral bioavailability (131%) with good exposure (AUC=36092 h*ng/mL), moderate half-life (4.28 h), and low protein binding (74.3% fraction unbound). The volume of distribution was moderate in both species (Vss = 1.32 L/kg in wistar han rat and 1.87 L/kg in beagle dog).

In cell culture, WNC0901 inhibited autophosphorylation of DNA-PKcs in HT29 cells irradiated with 10 Gy with an IC_{50} of 32.7 nM and robustly inhibited autophosphorylation in both U251 glioma and A549 lung cancer cell lines at 300 nM in combination with 5Gy. In a clonogenic assay, 5Gy irradiation (10% survival) combined with 100nM WNC0901 demonstrated modestly enhanced cell killing (1.5% survival), and maximal effects were seen at 300nM (0.04% survival, p<0.01). Similar radiosensitizing effects with 300 nM WNC0901 were seen in the A549 cell line (0.2% survival with combination compared to 19% with 5Gy alone, p<0.01).

CONCLUSIONS

In summary, WNC0901 inhibits DNA-PK kinase activity and provides potent radiosensitization in a GBM and lung cancer cell line. Future studies will assess the combination of WNC0901 and RT in GBM patient-derived xenograft models *in vivo*.

DNA-PK SIGNALING

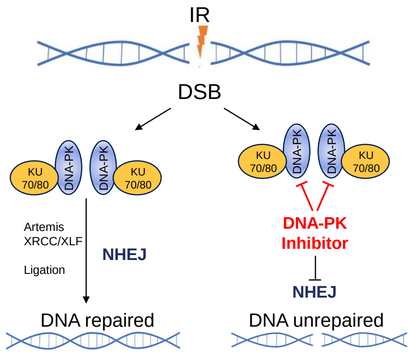


Figure 1. Mode of action of DNA-PK inhibitors[1,2]. DSB: DNA double-strand breaks; NHEJ: non-homologous end joining

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^{-6}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

Table 1: Potency, specificity and PK of Waynola DNA-PK inhibitor WNC0901.

TARGET INHIBITION

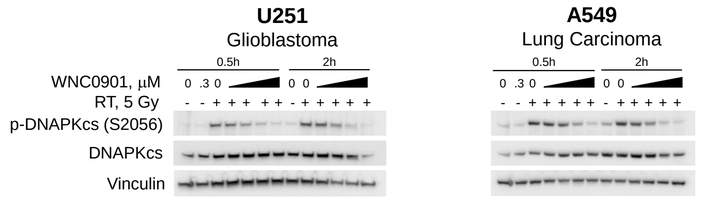
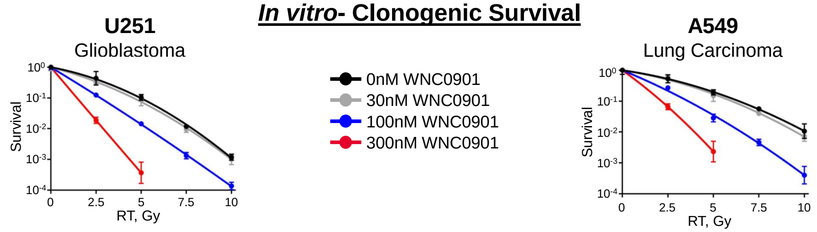


Figure 2. *In vitro* western blotting of U251 and A549 cells. Cells were treated with WNC0901, up to 1000nM, 30 min before 5Gy of irradiation, delivered by a PXI XRAD-320 cabinet irradiator. Proteins were harvested 0.5 and 2h after radiation.

RADIOSENSITIZING EFFECTS

A



B

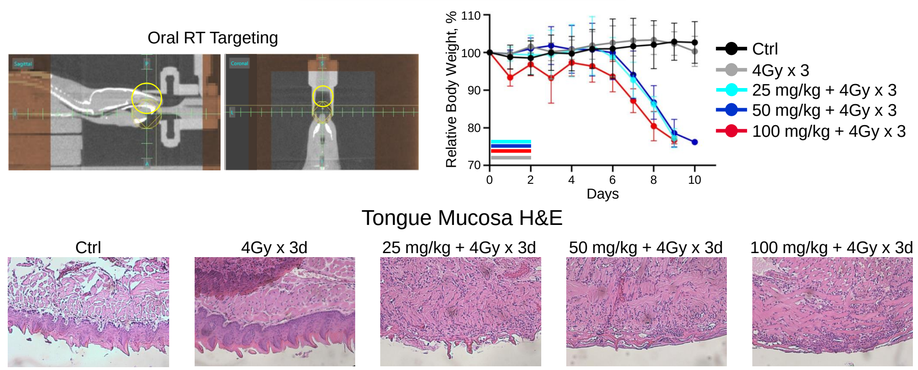


Figure 3. *In vitro* and *in vivo* radiosensitization by WNC0901. **A.** U251 or A549 cells were treated 4h after plating with a dose response of WNC0901 followed by increasing doses of radiation 30 min later. Drug was removed 24h later and colonies were counted on day 14. **B.** FVB female mice (n=5/group) were dosed for 3 consecutive days with two doses of WNC0901 given 7h apart and 4Gy irradiation, delivered to the oral mucosa 30 min after the first dose, using a SmART+ Stereotactic irradiator. Weights were taken daily, and mice were euthanized at moribund or day 10. Tongues were harvested at time of sacrifice for FFPE.

SUMMARY

- WNC0901 is a novel DNA-PK inhibitor that demonstrates adequate stability and bioavailability.
- Inhibition of pDNA-PKcs is achieved at concentrations of 300nM WNC0901 in glioblastoma and lung carcinoma cell lines and robustly reduces clonogenic survival when combined with radiation.
- In an *in vivo* oral mucositis model, WNC0901 radiosensitized mouse oral mucosa.

FUTURE DIRECTIONS

- Additional oral mucositis studies will be performed to define the minimally effective dose/schedule.
- Treatment with WNC0901 and RT will be tested in multiple established and PDX models.
- Further PD and PK parameters in study mice will be assessed.

REFERENCES, FUNDING, CONTACT

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3. Mayo Clinic Brain Tumor Patient-derived Xenograft National Resource

https://www.mayo.edu/research/labs/translational-neuro-oncology/mayo-clinic-brain-tumor-patient-derived-xenograft-national-resource/overview

National Brain Tumor Society

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Contact Ann Mladek Tuma with questions or comments
tuma.ann@mayo.edu

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