DUAL CK2/TNIK INHIBITOR, ON 108600 TARGETS CANCER STEM-LIKE CELLS AND REVERSES ACQUIRED PACLITAXEL RESISTANCE IN TRIPLE NEGATIVE BREAST CANCER CELLS

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INTRODUCTION & ABSTRACT

Triple negative breast cancer is associated with a poor prognosis and high frequency of recurrence, but because the molecular mechanisms are not well understood, there is a lack of targeted therapies. An undesirable consequence or limitation of existing therapies is the relapse state of the disease, which is highly resistant and metastatic and has been attributed to the presence of tumor-initiating stem cells (T-ICs). In patients with relapsed breast cancer, a $CD44^{high}/CD24^{-/low}$ antigenic phenotype has been shown to be enriched in T-ICs. Our previous data illustrated that a small molecule Casein Kinase 2 (CK2) inhibitor, ON 108600, potently inhibited the survival and growth of triple negative breast cancer cell lines and xenografts. To investigate whether ON 108600 has a similar inhibitory effect on T-ICs, we performed clonogenic survival assays with sorted and purified $CD44^{high}$ $CD24^{-/low}$ cells isolated from TNBC cell lines. ON 108600 potently inhibited the stem cell activity and self-renewal ability of these T-ICs isolated from TNBC cell lines.

Although paclitaxel treatment improves survival in TNBC, acquired resistance to paclitaxel is a common occurrence in TNBC. However, strategies that target paclitaxel resistant cells remain elusive. To investigate the molecular mechanisms underlying acquired paclitaxel-resistant in triple-negative breast cancer and evaluate the efficacy of ON 108600, we generated *in-vitro* chemoresistance models of paclitaxel resistance using well established TNBC cell lines; MDAMB-231 and BT-20. Drug-resistant cells were established by exposure to increasing concentrations of Paclitaxel, and resistance was validated by cell viability and colony formation. Paclitaxel-resistant MDAMB-231 and BT-20 (MDAMB-231_PTR and BT-20_PTR) cells exhibited ~1000 fold increase in resistance as compared to the parental cells. Significantly, paclitaxel resistant TNBC cells displayed a stem-like phenotype characterized by loss of epithelial differentiation markers (e-Cadherin, CD24) and a gain of mesenchymal transition markers (N-Cadherin, CD44, Oct4, Snail). Although CK2 subunit levels in resistant cells were unchanged, paclitaxel-resistant cells showed a marked upregulation of Traf2- and Nck-interacting kinase (TNIK), an activating kinase for T-cell factor-4 (TCF-4) and consequently a marked increase in β-catenin and Wnt target genes; Axin-2 and Cyclin D1. Kinase profiling studies indicate that ON 108600 targets not only protein kinase CK2, but also TNIK. Hence, to investigate if dual inhibition of CK2 and TNIK could result in inhibition of paclitaxel resistant T-ICs, we treated these resistant cells with ON 108600. Inhibition of CK2/TNIK with ON 108600 resulted in reduced cell growth and survival of paclitaxel resistant TNBC cells. Our results indicate that dual CK2/TNIK inhibition may be an effective way to overcome paclitaxel resistance in TNBC.

RESULTS

ON 108600 A POTENT CK2/TNIK INHIBITOR SUPPRESSES TNBC CELL GROWTH IN VITRO

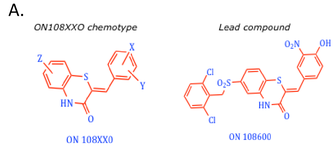

Table 1.

KINASE	IC_{50} (μM)
CK2α1	0.05
CK2α2	0.005
TNIK	0.005
DYRK1A	0.016
DYRK1B	0.007

Figure **A**. ON 108XXO chemotype and ON 108600 structure. **B**. ON 108600 potently inhibits kinases Traf2- and Nck-interacting kinase (TNIK) and Casein kinase 2 (CK2) catalytic subunits. **C**. Viability 11 breast cancer cell lines 72 hours after ON 108600 treatment (250 nM) relative to vehicle control assessed by Cell-Titer Blue.

Table 1. Kinase inhibitory profile of ON 108600.

BASAL-LIKE TNBC CELLS AND THEIR T-ICS ARE SELECTIVELY SENSITIVE TO ON 108600 TREATMENT

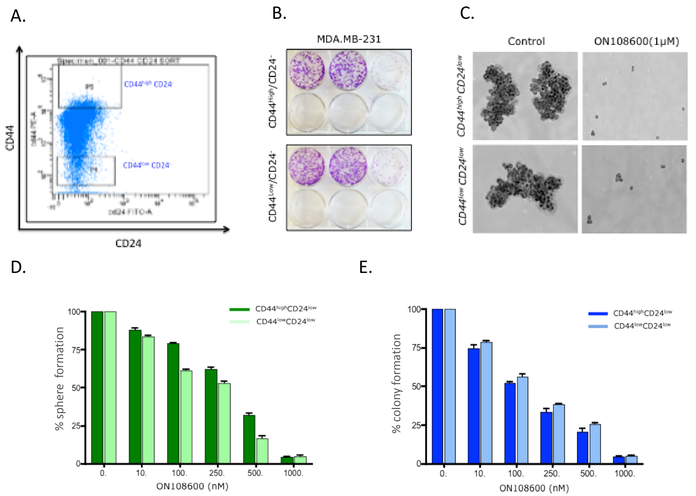

Figure **A**. Expression patterns of cell surface antigens, CD24 and CD44 in MDAMB-231 TNBC cells were analyzed by FACS. **B**. Purified $CD44^{high}$ $CD24^{-/low}$ and $CD44^{low}$ $CD24^{-/low}$ cells from MDAMB-231, BT20 and Hs578T were purified and treated with various concentrations of ON 108600 for 72 hours, washed and allowed to grow for 7 days in drug-free growth medium and the colonies were stained with crystal violet. **C**. Alternatively purified cells were plated in ultra-low attachment plates containing mamosphere-forming medium and treated with ON 108600 for 72 hours. **D,E**. Schematic representation of inhibition of mamosphere forming capacity and colony forming ability of purified $CD44^{high}$ $CD24^{-/low}$ cells from TNBC/Basal-B cell lines after treatment with DMSO or ON 108600.

CHEMORESISTANT (PACLITAXEL) RESISTANT TNBC CELLS HAVE A BCSC LIKE PHENOTYPE

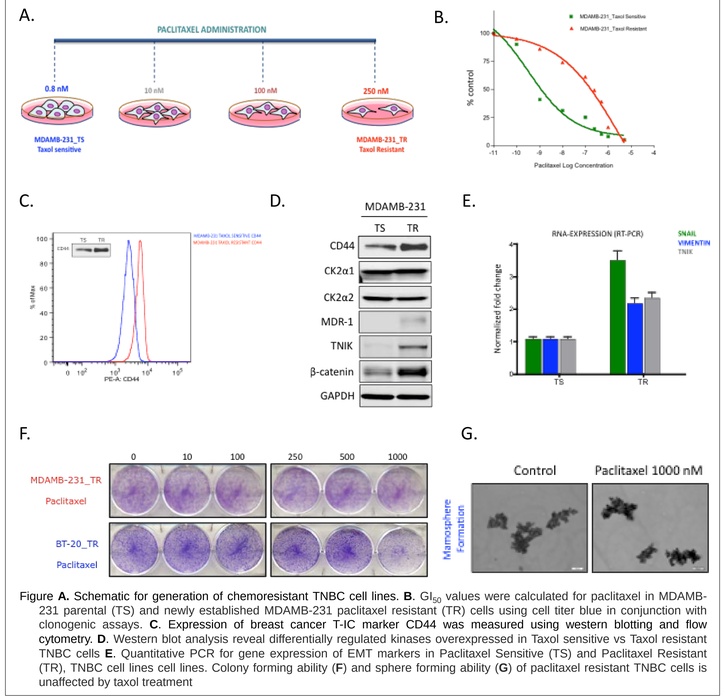

Figure **A.** Schematic for generation of chemoresistant TNBC cell lines. **B**. GI_{50} values were calculated for paclitaxel in MDAMB-231 parental (TS) and newly established MDAMB-231 paclitaxel resistant (TR) cells using cell titer blue in conjunction with clonogenic assays. **C**. Expression of breast cancer T-IC marker CD44 was measured using western blotting and flow cytometry. **D**. Western blot analysis reveal differentially regulated kinases overexpressed in Taxol sensitive vs Taxol resistant TNBC cells **E**. Quantitative PCR for gene expression of EMT markers in Paclitaxel Sensitive (TS) and Paclitaxel Resistant (TR), TNBC cell lines cell lines. Colony forming ability (**F**) and sphere forming ability (**G**) of paclitaxel resistant TNBC cells is unaffected by taxol treatment

PACLITAXEL RESISTANT TNBC CELLS ARE SENSITIVE TO CK2/TNIK INHIBITOR ON 108600

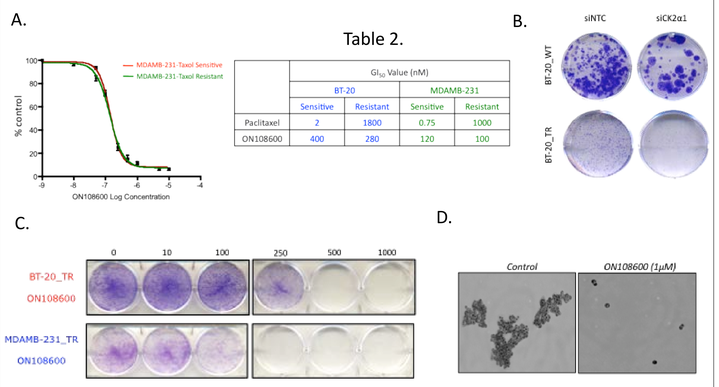

Table 2.

	GI_{50} Value (nM)			
	BT-20		MDAMB-231	
	Sensitive	Resistant	Sensitive	Resistant
Paclitaxel	2	1800	0.75	1000
ON108600	400	280	120	100

Figure **A**. Paclitaxel resistant TNBC cell lines are equally sensitive to ON 108600 treatment. **B**. Colony forming potential of taxol resistant TNBC cells is inhibited by CK2 RNAi (25 nM). **C, D**. Inhibition of clonogenic and mamosphere forming ability of taxol resistant cells after treatment with ON 108600.

Table 2. Growth inhibitory profile of ON 108600 and Paclitaxel in sensitive and resistant TNBC cell lines.

CONCLUSION

- ON 108600 is a potent CK2/TNIK inhibitor with inhibitory activity towards triple negative breast cancer cell lines.
- Basal-like TNBC cells are enriched in $CD44^{high}$ $CD24^{-/low}$ tumor initiating breast cancer stem cells.
- ON 108600 potently inhibits stem cell activity and self renewal ability of these T-ICs populations.
- Paclitaxel resistant TNBC cells display a breast cancer stem-cell like phenotype (loss of epithelial differentiation markers and gain of epithelial-mesenchymal transition markers.
- Paclitaxel resistant TNBC cells show marked upregulation of kinase TNIK and the β-catenin pathway.
- CK2 inhibitor ON 108600 is highly effective in killing paclitaxel resistant TNBC cells.
- ON 108600 potently inhibits the clonogenic and mamosphere forming ability of paclitaxel resistant TNBC cells.
- ON 108600 is a novel small molecule inhibitor, which overrides paclitaxel resistance in TNBC.

FUTURE DIRECTIONS

- To investigate the role of kinases CK2 and TNIK in acquired paclitaxel resistance in triple negative breast cancer.
- To further interrogate the significance of CK2 and TNIK in published datasets derived from chemotherapy resistant human triple negative breast cancer tissues.
- To extend and validate these models in patient derived tumor xenograft models.
- To test the efficacy of ON 108600 in patient derived mouse models.
- To identify novel mechanisms by which CK2 regulates chemotherapeutic resistance.