#1287

The Dual IAP Antagonist, ASTX660, Increases The Anti-tumor Activity of Paclitaxel in TNBC Preclinical Models In Vivo

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INTRODUCTION

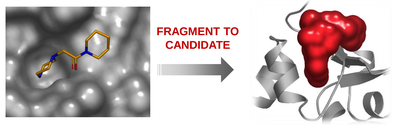
Paclitaxel-mediated secretion of inflammatory mediators, including TNFα, potentially creates paracrine and autocrine loops that can contribute to survival and reduction of paclitaxel-induced apoptosis in cancer cells.

One of the mechanisms of cancer cell survival is the expression of inhibitor of apoptosis proteins (IAPs). Cellular IAP (cIAP) is involved in inflammatory pro-survival NF-κB activation, blocking the activation of the effector caspases 3 and 7, while X-linked IAP (XIAP) directly binds the effector caspases 3, 7 and 9, inhibiting the full activation of the apoptosis pathway.

ASTX660, a fragment-derived small molecule that is orally bioavailable, is a dual antagonist of cIAP and XIAP (Chessari 2014). Its inhibitory activity has been demonstrated in preclinical models of melanoma and other types of cancer, in which inflammation was present. It is currently being investigated in a single-agent Phase I/II clinical trial in patients with advanced solid tumors and lymphomas (NCT02503423).

Here, we characterize the activity of ASTX660 in preclinical models of triple-negative breast cancer (TNBC) as a single agent and in combination with paclitaxel whose inflammatory properties are hypothesized to sensitize the cells to ASTX660.

ASTX660 derived from fragment-based drug discovery



FRAGMENT TO CANDIDATE

- Balanced cIAP1/XIAP profile
- Non peptidomimetic
- Non alanine warhead
- Oral

EFFECTS OF ASTX660 ON TNBC CELL LINES IN VITRO

ASTX660 treatment reduces viability of TNBC cells

In vitro cell line screening

Cell line	Maximum inhibition (% of DMSO control) ASTX660	ASTX660 + TNFα
MDA-MB-231	79	84
HCC38	52	97
BT-549	37	89
HS578T	18	78
EMT-6	12	97
HCC1806	0	96
DU4475	1	91
HCC1395	0	68
MDA-MB-453	0	66
MDA-MB-468	0	39
BT-20	0	38
MDA-MB-157	0	33
MDA-MB-436	0	32
HCC70	9	25
HCC2157	1	22
HCC1187	8	19
HCC1599	8	17
HCC1143	9	16
HCC1569	0	4
MX-1	5	0
HCC1937	0	0

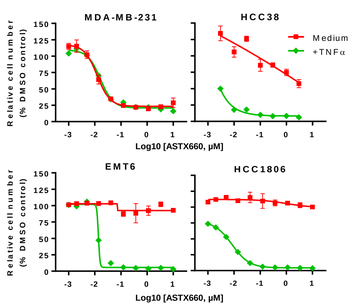


Table 1: Summary of TNBC cell line screening. Viability of TNBC cells after 72-hour treatment with ASTX660 alone or in the presence of 1 ng/ml rhTNFα was assessed by CellTitre Glo assay. TNFα was added to demonstrate that ASTX660 converts inflammatory signal into cell death. Figure 1: Representative IC_{50} curves of cell lines that showed sensitivity towards ASTX660.

ANTAGONISM OF XIAP & cIAP IN VITRO

ASTX660 reduces XIAP interaction with Caspase 9 and causes rapid degradation of cIAP1

Compound	Target cellular activity (EC_{50}, nM) XIAP	cIAP1	*XIAP/cIAP EC_{50} ratio*
ASTX660	2.9	0.22	*13*
CUDC-427	10	0.04	*250*
LCL-161	35	0.4	*88*
Debio-1143	35	0.99	*35*
Birinapant	23	0.23	*100*

Table 2: Summary of ASTX660 XIAP and cIAP1 antagonistic activities compared to other IAP antagonists. Inhibition of XIAP:Caspase 9 interaction was measured by treating HEK293 cells, engineered to stably express XIAP and caspase 9, and co-immunoprecipitating the proteins. Degradation of cIAP1 was measured in the lysates of treated MDA-MD-231 cells.

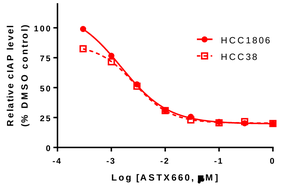
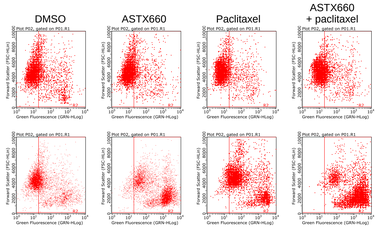


Figure 2: Degradation of cIAP1 in HCC38 and HCC1806 cells after a 2-hour treatment with ASTX660 were detected by MSD, demonstrating cIAP antagonistic activity of ASTX660.

HCC1806 cells treated with ASTX660, paclitaxel and TNFα undergo apoptosis



	% positively-stained cells (median fluorescence) DMSO	ASTX660	Paclitaxel	ASTX660 + Paclitaxel
Medium	25 (11)	20 (10)	14 (7)	26 (12)
TNFα	51 (19)	95 (880)	92 (55)	99 (1626)

Figure 3 & Table 3: FACS analysis of in HCC1806 cells treated with various combinations of ASTX660 (1 μM), paclitaxel (1 μM) and rhTNFα (1 ng/ml) for 6 hours. Cells were stained for caspase 3/7 activity (green fluorescence) and positively-stained population increased when treated with all agents.

IN VIVO ANTI-tumor ACTIVITY OF ASTX660 IN TNBC MODELS

ASTX660 monotherapy inhibits tumor growth in vivo

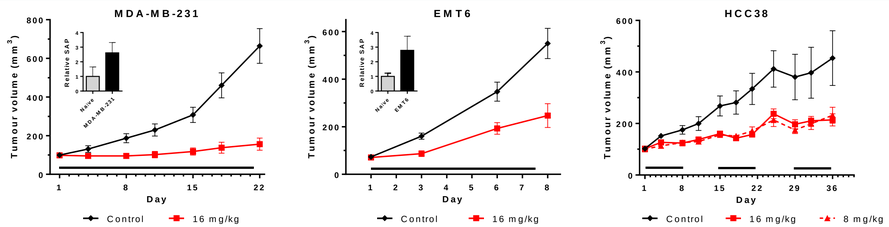


Figure 4: TNBC xenograft studies showing anti-tumor activity of ASTX660. Mice bearing TNBC tumors were orally treated with 16 or 8 mg/kg of ASTX660 lactate dissolved in water once a day for the indicated period. All treatments were well-tolerated. Serum amyloid P component (SAP) was measured in the plasma of untreated MDA-MB-231 or EMT6 tumor-bearing mice by ELISA (inset).

Paclitaxel increases inflammatory markers in mice

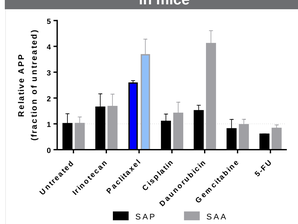


Figure 5A: Two acute-phase proteins, serum SAP and serum amyloid A (SAA) in plasma of BALB/c SCID mice were elevated 24 hours after dosing paclitaxel. Drugs were given intraperitoneally (ip) at standard doses: irinotecan (100 mg/kg), paclitaxel (20 mg/kg), cisplatin (4 mg/kg), daunorubicin (6 mg/kg), gemcitabine (80 mg/kg) and 5-FU (65 mg/kg). All data were normalized to the mean of untreated samples.

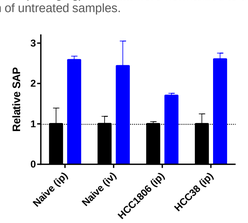
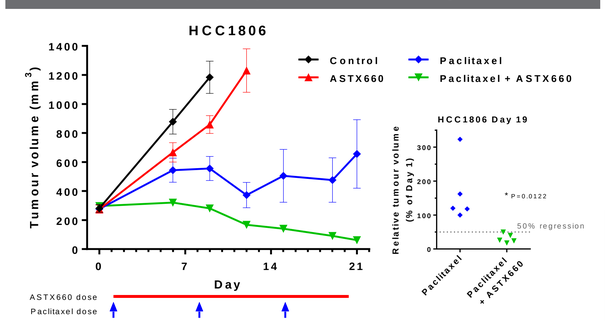


Figure 5B: SAP increase was found in naïve and TNBC tumor-bearing animals 24 hours after the dosing by intraperitoneal (ip) or intravenous (iv) route. All data were normalised to the mean of untreated naïve or tumor-bearing samples.

Combination of ASTX660 and paclitaxel enhanced tumor growth inhibition



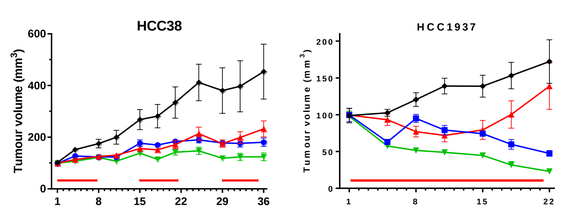


Figure 6: Combination studies demonstrating the effects of ASTX660 and paclitaxel in vivo. Mice bearing TNBC tumors were treated with paclitaxel once a week, ASTX660 once daily for the indicated period or combination of both. ASTX660 was dissolved in water, paclitaxel in 10% cremophor, 10% ethanol and 80% saline. HCC1806 study was performed in BALB/c nude mice with paclitaxel 20 mg/kg iv & ASTX660 25 mg/kg; HCC38 & HCC1937 in BALB/c SCID mice with paclitaxel 20 mg/kg ip & ASTX660 8 mg/kg. The two ASTX660 dose levels gave similar systemic exposure.

ASTX660 antagonises XIAP & cIAP in vivo & causes apoptosis

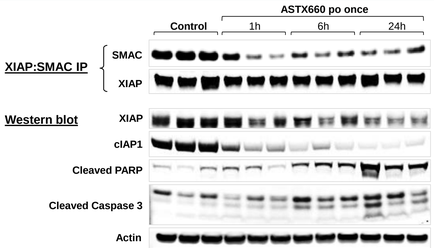


Figure 7: Western blots showing IAP antagonism and cleavage of apoptotic proteins. XIAP antagonism (XIAP:SMAC co-immunoprecipitation with XIAP antibody), degradation of cIAP1, reduction in XIAP and cleavage of PARP and caspase 3 (Western blot) were detected in MDA-MB-231 xenograft in BALB/c nude mice after a single dose of ASTX660 hydrochloride at 30 mg/kg.

Degradation of cIAP1 by ASTX660 is enhanced in combination with paclitaxel

Figure 8: Western blot showing cIAP1 degradation of cIAP1 detected in HCC1806 tumors treated with ASTX660 and/or paclitaxel. Tumor-bearing BALB/c nude mice treated for 3 days with ASTX660 lactate (Days 1-3), paclitaxel (20 mg/kg iv, Day 1) or combination and tumors were collected on Day 4.

CONCLUSIONS

- ASTX660 is effective against 40% of TNBC cell lines in vitro in the presence of TNFα. TNBC cells treated with ASTX660 in the presence of TNFα undergoes apoptosis via caspase cleavage following IAP antagonism.
- TNBC tumors that have inflammatory environment showed sensitivity towards ASTX660 in vivo.
- Paclitaxel not only has anti-tumor activity but also increases systemic inflammatory markers. Combination of ASTX660 and paclitaxel resulted in enhanced tumor growth inhibition over paclitaxel alone.
- Combining IAP antagonist with another therapeutic agent that provides inflammatory tumor microenvironment could be a valid therapeutic approach.
- ASTX660 is currently in Phase I/II clinical trial in patients with advanced solid tumors and lymphomas (NCT02503423).

Reference:
Chessari et al, (2014). Induction of apoptosis with a novel dual cIAP1/XIAP antagonist in models of melanoma. European Journal of Cancer 50:122.

TAIHO PHARMA

astex pharmaceuticals

Poster presented at AACR 2016, 16-20th April 2016, New Orleans, USA (Poster available to download from Astex's website at www.astx.com)