AACR poster #1439

Increasing potency of anticancer drugs through SORT1+ Technology™: A new targeted approach for the treatment of ovarian and endometrial cancers

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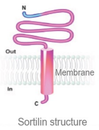
Introduction

SORTILIN (SORT1) RECEPTOR IN CANCER

- Sortilin receptors are preferentially expressed in many cancers compared to healthy tissues, which makes it an attractive target for cancer drug development.
- Transmembrane scavenger receptor involved in import-export of peptides into the cell via the endosomal/lysosomal pathway (cellular shuttle system).
- Ideal candidate for internalization of peptide-drug conjugates (PDC's).
- Sortilin expression increases as a function of tumor grade (I to IV) and is associated with poor prognosis and decreased survival in different cancers.
- Known sortilin expression in various tumor types:

TNBC	59%
Invasive ductal breast	79%
Ovarian (OvCa)	>90%
Endometrial (EC)	>90%
Colorectal (CRC)	30-40%
Pancreatic	30-50%
Melanoma	>90%



Sortilin structure

BACKGROUND

Ovarian cancer

- One of the most lethal gynecologic malignancies, often diagnosed at late stage.
- 90% of OvCa cases are malignant epithelial tumors.
- There are five main subtypes of epithelial ovarian cancer (EOC): high-grade serous carcinoma (HGSC), clear cell carcinoma (CCC), endometrioid carcinoma (EC), mucinous carcinoma (MC), and low-grade serous carcinoma (LGSC) accounting for 68%, 12%, 11%, 3% and 3% of EOCs, respectively.
- HGSCs often manifest at an advanced stage and are biologically aggressive with up to 85% of patients with ovarian serous carcinoma presenting with widespread peritoneal metastases. Up to 80% of HGSCs show an initial response to platinum-based chemotherapy, but about 70% demonstrate recurrence. Despite significant advances in the treatment of ovarian cancer, relapse is observed in 40-85% of patients in stages II-IV after primary therapy.

Endometrial Cancer

- Endometrial cancer is the most common gynecologic malignancy. It is the 4th most common cancer in women in the United States after breast, lung, and colorectal cancers and 6th cause of cancer death in women in the USA. (Death rate per 100,000 population has increased more than 100% during the past 20 years and 8% since 2008 (Sorosky, 2012)).
- Projections from the ACS for 2021 estimated 66,570 new cases of cancer of the body of the uterus (uterine body or corpus) will be diagnosed and about 12,940 women will die (ACS, 2021).
- From a clinical perspective, incidence of EC is rapidly increasing worldwide, with highest disease burden in North America and Western Europe. Although prognosis remains good for patients diagnosed with early-stage EC, recurrent or metastatic patients have few options, and the median overall survival is short.

SORT1+ TECHNOLOGY™ PLATFORM

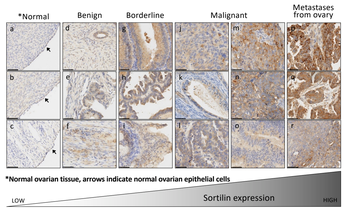
- **SORT1+ Technology™** is an innovative oncology platform consisting of novel peptides which target the SORT 1 receptor
- Targeting sortilin receptors with these peptide-drug conjugates (PDC's) leads to receptor-mediated internalization (endocytosis) of well-established anti-cancer agents (e.g., docetaxel, doxorubicin, curcumin) that are attached to the novel proprietary peptide
- Once inside the cancer cells, active drug is released from the peptide and exerts its cytotoxic effect directly on the cancer cell, sparing normal cells from toxicity
- Versatile and flexible conjugation strategies achieve different ratios of drug to peptide

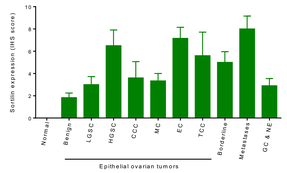
Results

SORTILIN STAINING IN TISSUE MICROARRAYS (TMA)

A. Ovarian cancers

- IHC shows high expression of sortilin in ovarian cancers.

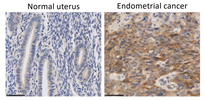


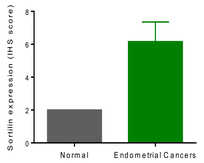


Legend (n = number of biopsies)
Normal (20)
Benign (18)
LGSC: low-grade serous carcinoma (21)
HGSC: high-grade serous carcinoma (6)
CCC: clear cell carcinoma (5)
MC: mucinous carcinoma (3)
EC: endometrioid carcinoma (13)
TCC: transitional cell carcinoma (5)
Borderline (11)
Metastases (10)
GC & NE: germ cell & other non-epithelial (20)

B. Endometrial cancers

- IHC shows high expression of sortilin in endometrial cancers.

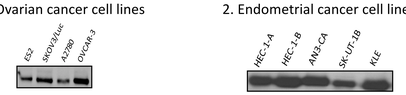




Legend (n = number of biopsies)
Normal uterus (2)
Endometrial cancers (12)

C. Sortilin detection in cancer cell line models

1. Ovarian cancer cell lines
2. Endometrial cancer cell lines



Sortilin Immunodetection in Human Cancer Cell Lines by Western Blotting. *Sortilin expression was evaluated by Western blot analysis of cell lysates (20 µg) from cancer cell lines.* ***1)*** *Human ovarian cancer cell lines and* ***2)*** *Human endometrial cancer cell lines.*

Results (cont'd)

CELL PROLIFERATION AND PEPTIDE INTERNALIZATION

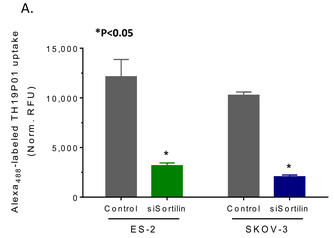
A. Anti-proliferation

Cancer cells	IC_{50} (nM) Docetaxel	IC_{50} (nM) TH1902
ES-2 (ovarian cancer cells)	7.4 ± 2.2	8.1 ± 3.0
AN3-CA (endometrial cancer cells)	0.54 ± 0.15	0.46 ± 0.06
MDA-MB-231 (Triple-negative breast cancer)	0.38 ± 0.12	0.54 ± 0.11

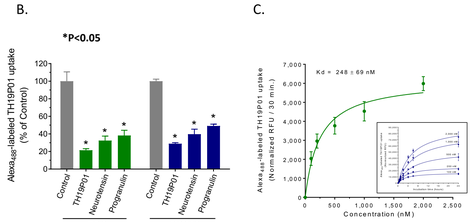
In the presence of TH1902, anti-proliferative activities were seen at low concentrations in ovarian, endometrial and TNBC cell lines. This demonstrates that conjugation of docetaxel to the peptide, TH19P01, does not compromise its anticancer potency.

B. Sortilin-mediated Internalization

Effect of sortilin gene silencing (siRNA)



Pharmacological inhibition with sortilin ligands



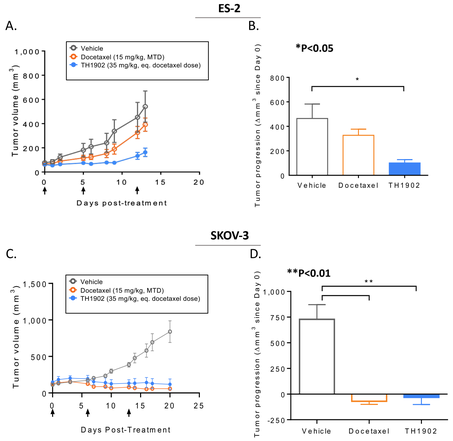
Involvement of sortilin in the internalization of $Alexa_{488}$-labeled TH19P01 in ES-2 and SKOV-3 ovarian cancer cells. A) Uptake of $Alexa_{488}$-labeled TH19P01 (200 nM) was measured after 2 h in cells where SORT1 was silenced. **B)** Uptake of $Alexa_{488}$-labeled TH19P01 (200 nM) was measured after 2 h incubation in ES-2 and SKOV-3 ovarian cancer cells in the absence or presence of excess unlabeled TH19P01 (50 µM), neurotensin (10 µM) or progranulin (1 nM). **C)** Uptake time-course of $Alexa_{488}$-labeled TH19P01 in sortilin-positive ES-2 cells with increasing concentrations (inner curves set). Uptake values in the linear phase were then plotted according to $Alexa_{488}$-labeled TH19P01 concentrations in order to calculate kinetic parameters using a one site-specific binding analysis with GraphPad Prism Software (outer curve set).

Results (cont'd)

IN VIVO PROOF OF PRINCIPLE OF SORT1+ TECHNOLOGY™ PLATFORM INCREASED EFFICACY WITH IMPROVED SAFETY

A. Ovarian s.c. xenograft tumor models

- **Better and sustained efficacy** with TH1902-treated mice at a dose equivalent to the MTD of docetaxel in OvCa and EC models.
- **Significant improvement of efficacy** with TH1902 even when administered at lower dose (1/4 of the equivalent MTD dose of docetaxel).
- **Higher cumulative injected dose** for TH1902 (up to 2-fold).

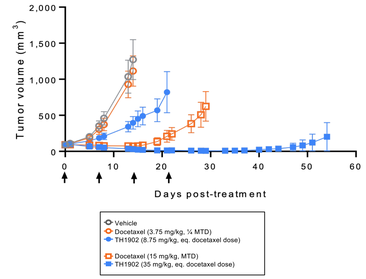


Effects of TH1902 on Ovarian Tumor Xenograft Volume and Progression. *Mice bearing ovarian tumors were treated with TH1902, docetaxel at an equivalent dose or with vehicle.* ***A)*** *ES-2 tumor volume measurements and* ***B)*** *tumor progression at Day 13 post-treatment or* ***C)*** *SKOV-3/Luc tumor volume measurements and* ***D)*** *tumor progression at Day 20 post-treatment. Tumor volumes were measured at each point shown. Arrows indicate dates of IV injections for all test articles.*

Results (cont'd)

STRONG INHIBITION OF TUMOR GROWTH (AN3-CA Xenografts)

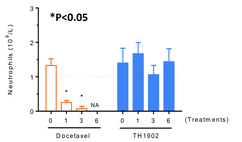
B. Endometrial s.c. xenograft tumor model



Effects of TH1902 on Endometrial Tumor Xenograft Volume. *Mice bearing AN3-CA endometrial tumors were treated with TH1902, docetaxel or the vehicle. AN3-CA tumor volumes measurements following IV bolus injection of TH1902 or docetaxel at equivalent docetaxel MTD or 1/4 MTD doses. Arrows indicate dates of IV injections for all test articles.*

C. Absence of neutropenia in athymic mice

- **Absence of neutropenia** after 6 consecutive treatments of TH1902 at an equivalent dose of MTD docetaxel (15 mg/kg/week, 3 cycles) in athymic mice.
- In contrast, 1 treatment with unconjugated Docetaxel strongly reduced neutrophil counts.



Conclusions

- SORT1+ Technology™ is an innovative, flexible platform consisting of novel peptides that target the sortilin receptor (SORT1).
- The proprietary peptide, TH19P01, can be conjugated to well characterized anticancer agents, such as docetaxel (TH1902) and doxorubicin (TH1904) for which efficacy and safety have been well established in the clinical setting.
- TH1902 peptide-drug conjugate is internalized via a sortilin dependent endocytic mechanism of action. Sortilin receptor is preferentially expressed in cancer cells.
- TH1902 has demonstrated antiproliferative activity across multiple tumor types and conjugation of docetaxel to the proprietary peptide (TH19P01) does not compromise its anticancer potency.
- TH1902 demonstrated better and sustained efficacy at doses equivalent to the MTD of docetaxel in ovarian and endometrial s.c. xenograft tumor models. Improved efficacy was also seen at doses lower than MTD docetaxel (1/4 of the equivalent MTD). Safety profile (neutropenia) was also significantly improved over that seen with docetaxel. This could be important clinically as neutropenia is a dose limiting toxicity that affects dosing, efficacy and tolerability.
- This preclinical data demonstrates that SORT1+ Technology™ is a precision medicine approach for delivery of established anticancer drugs directly inside the tumor cells, thereby optimizing efficacy, limiting toxicity and improving the therapeutic window of the cytotoxic overall.
- Ovarian and endometrial cancers have poor prognosis and survival outcomes clinically, and TH1902 has demonstrated benefit in both tumor models.

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