AACR poster # 1853

TH1902, a docetaxel peptide-drug conjugate targeting SORT1, inhibits tumor growth of human cancer stem-like cells (CD133+) from both triple-negative breast cancers and ovarian cancers

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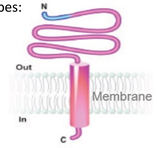
Introduction

SORTILIN (SORT1) RECEPTOR IN CANCER

- Sortilin receptor is 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 (PDCs).
- 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%



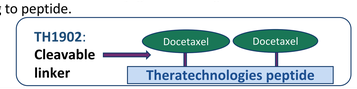
Sortilin structure

BACKGROUND

- Cancer Stem Cells (CSCs) contribute immensely to the carcinogenesis in the clinic.
- CSCs are heterogeneous, involving various cellular markers and regulatory signaling pathways.
- CSCs exhibit several phenotypes such as migration, invasion, self-renewal, and chemotherapy as well as radiotherapy resistance.
- CD133 is a known stem cell marker.
- CD133+ cancer cells are reported to participate in vasculogenic mimicry (VM) processes reflecting the plasticity of aggressive tumor cells.
- SORT1 is implicated in the formation of VM structures.
- Interestingly, TH1902 showed anti-VM properties at low concentrations (Charfi *et al*., Front Oncol 2021).
- Commercial human stem-like cells from triple-negative breast cancer (hTNBCSC) or ovarian cancer (hOvCSC) were purchased from Celprogen and maintained according to the manufacturer's instructions.
- These CSCs are positive for CD133, CD44, SSEA3/4, Oct4, Tumorigenicity (<1000 cells), Alkaline Phosphatase, Aldehyde Dehydrogenase, Telomerase.

SORT1+ TECHNOLOGY™ PLATFORM

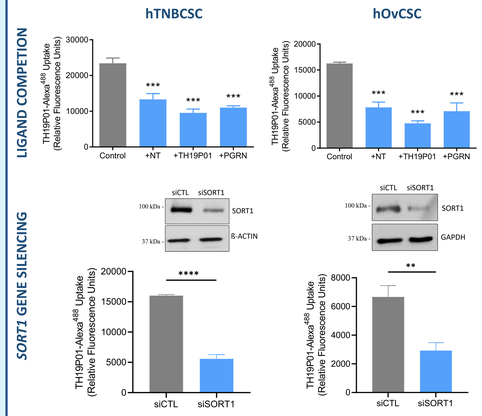
- **SORT1+ Technology™** is an innovative oncology platform consisting of novel peptides which target the SORT1 receptor.
- Exploiting SORT1 function with these peptide-drug conjugates (PDCs) leads to rapid receptor-mediated internalization (endocytosis) of well-established anti-cancer agents (e.g., 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-MEDIATED INTERNALIZATION AND APOPTOSIS

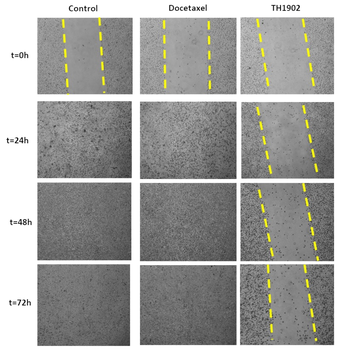
A. Peptide uptake (SORT1 ligand competition and gene silencing)



**P<0.01, ***P<0.001 , ****P<0.0001

- Both cancer stem cell lines express the sortilin receptor.
- The internalization of the fluorescently labeled TH19P01 is inhibited by either sortilin ligands or gene silencing.

B. TH1902 inhibits hTNBCSCs migration/invasion



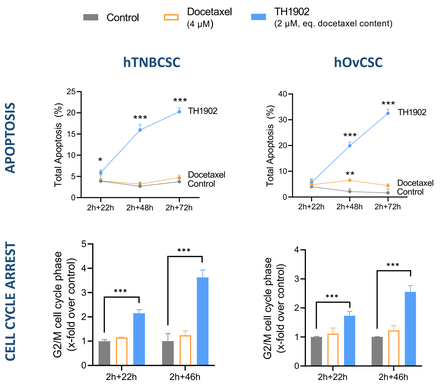
Cells were treated with 2µM of docetaxel or 1µM of TH1902 (equivalent docetaxel content)

- TH1902 inhibits the migration/invasion of human TNBC stem cells for a prolonged period (72h) whereas docetaxel (at equivalent docetaxel concentration) has no observable effect.

Results (cont'd)

SORTILIN-MEDIATED INTERNALIZATION AND APOPTOSIS

C. TH1902 induction of apoptosis and cell cycle arrest in docetaxel-resistant CSC lines



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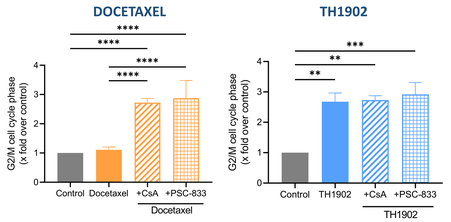
- At equivalent docetaxel content, TH1902 is able to induce significant apoptosis and cell cycle arrest into both cancer stem cell lines whereas docetaxel had minimal effect.

D. TH1902 bypasses the P-gp efflux pump

- These hTNBCSCs are known to be resistant to docetaxel and doxorubicin.
- These CSCs express SORT1 and P-gp (MDR1).



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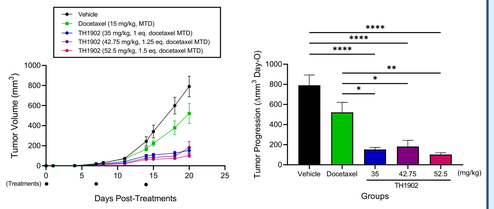
- In contrast to docetaxel, TH1902 can induce G2/M cell cycle arrest.
- P-gp inhibitors (Cyclosporin A and PSC-833) can restore the cell cycle arrest induced by docetaxel whereas TH1902 is unaffected.
- These results suggest that TH1902 bypasses the P-gp efflux pump.

Results (cont'd)

IN VIVO PROOF OF PRINCIPLE OF SORT1+ TECHNOLOGY™ PLATFORM (INCREASED EFFICACY ACROSS CSC TUMOR MODELS)

A. Human TNBC CSC s.c. xenograft tumor models



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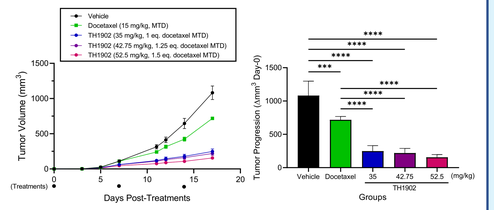
B. Human Ovarian CSC s.c. xenograft tumor models



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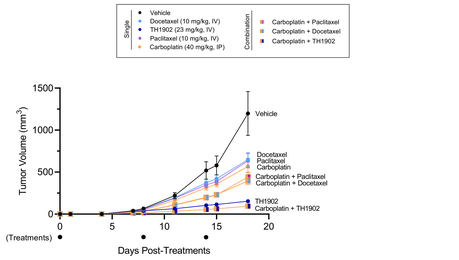
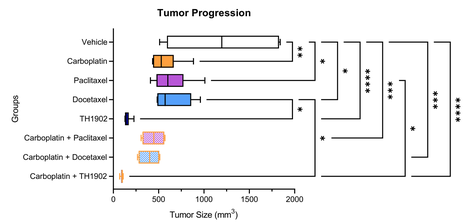
- **Significant improvement of efficacy** with TH1902 at a dose equivalent to the MTD of docetaxel in both cancer stem cells xenograft models.

Results (cont'd)

IN VIVO PROOF OF PRINCIPLE OF SORT1+ TECHNOLOGY™ PLATFORM (INCREASED EFFICACY ACROSS CSC TUMOR MODELS)

C. TH1902 and carboplatin combination (hOvCSC model)





*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

- **Better efficacy** in TH1902-treated mice at a dose equivalent to docetaxel and paclitaxel and in TH1902 –carboplatin combination treated mice.

Conclusions

- **SORT1+ Technology™** is an innovative, flexible platform consisting of novel peptides that target the sortilin receptor (SORT1).
- Sortilin is highly expressed in various cancer cells and cancer stem cells.
- The proprietary peptide, TH19P01, can be conjugated to well characterized anticancer agents, such as docetaxel (TH1902) and doxorubicin (TH1904).
- TH1902 peptide-drug conjugate is internalized via a sortilin-dependent endocytic mechanism.
- Once internalized, TH1902 induces apoptosis to a greater extent than docetaxel alone in breast and ovarian CSCs.
- As a single agent, TH1902 demonstrated better efficacy at doses equivalent to docetaxel in both breast and ovarian CSC xenograft tumor models.
- Better efficacy was also observed in the hOVCSC xenograft tumor model for the TH1902-carboplatin combination compared to paclitaxel- or docetaxel-carboplatin standard of care combinations.
- This preclinical data demonstrates that **SORT1+ Technology™** is a novel therapeutical approach for delivery of established anticancer drugs to tumor cells, thereby optimizing efficacy and improving the therapeutic window of the cytotoxic.

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