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# Receptor-Mediated Chemotherapy Using a New Docetaxel-Peptide Conjugate for Sortilin-Positive Triple-Negative Breast Cancer

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## Abstract

**Background:** Triple-negative breast cancer (TNBC) is a heterogeneous disease which still lacks defined molecular biomarkers. In the last decade, targeting of specific gene/protein molecular signature of tumors has emerged amongst the optimal anticancer strategies. Recently, increased expression of the sortilin (SORT1) receptor has been reported in TNBC patients. Given SORT1 functions in protein internalization, sorting and trafficking, we developed a novel peptide-anticancer drug conjugation platform to target SORT1-positive cancers by linking docetaxel to a peptide (TH19P01) that specifically targets SORT1. Here we are reporting the results obtained in TNBC.

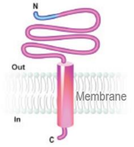
**Results:** In MDA-MB-231 cells, the docetaxel-TH19P01 peptide conjugate (TH1902) exerted potent anti-proliferative and anti-migratory activities *in vitro*. TH1902 triggered faster and higher cell death mechanisms than did free docetaxel alone. The apoptotic and anti-migratory effects were reversed by the SORT1 ligands neurotensin and progranulin, or by siRNA-mediated silencing of SORT1. *In vivo*, TH1902 exhibited greater tumor regression ability with prolonged survival in a murine MDA-MB-231 xenograft TNBC tumor model than did docetaxel. Hematotoxic assays revealed that neutrophil counts decreased significantly in docetaxel-treated mice at MTD after 3 cycles, whereas neutrophil levels remained within normal limits in TH1902-treated mice (at a dose equivalent to the docetaxel) and remained normal even after 6 TH1902 injections. Preliminary pharmacokinetics for TH1902 were also evaluated in normal CD-1 mice. Plasma concentrations of TH1902 and of released docetaxel were measured by UPLC/MS after a single IV bolus injection at 50 mg/kg. Plasma concentrations of released docetaxel revealed that most docetaxel remained associated with the peptide over the time period analyzed. Low levels of free docetaxel in mouse plasma may partially explain the absence of neutropenia in mice treated with TH1902.

**Conclusions:** We demonstrated that TH1902 is specifically internalized through a SORT1 receptor-mediated mechanism. This property allows specific targeting of SORT1-positive breast cancer cells. The docetaxel conjugation increases its efficacy and decreases the toxicity compared to free docetaxel molecules, making TH1902 a promising, novel therapy for the treatment of TNBC.

## Introduction

### SORTILIN IN CANCER

- Receptor involved in cancer cell survival and progression
- Specialized in the internalization and trafficking of its ligands (ex. neurotensin, progranulin)
- Associated with breast cancer aggressiveness:
  - 79% of invasive ductal breast cancer
  - 59% of TNBC
  - Increased expression as function of tumor grade (I to IV)
- Other cancers overexpressing sortilin: ovarian, endometrial, lung, melanoma, colorectal and pancreatic cancers



Sortilin structure

### TRIPLE-NEGATIVE BREAST CANCER (TNBC)

It is estimated that in 2019:

- Breast cancer is the **most common** cancer and the **first leading cause** of cancer-related deaths among women
- **2.1 million women** worldwide will be diagnosed with the disease
- TNBC accounts for **15-20%** of breast cancers worldwide and remains the deadliest subgroup of breast cancer
- TNBC is considered **more aggressive** than other breast cancers and **more difficult to treat**
- Amongst women treated for TNBC, **42%** will have rapid relapses with a peak 3 years after diagnosis
- Currently, **no targeted therapies** are approved for the treatment of TNBC; thus, surgery, anthracycline-, taxane-based chemotherapy, and radiation therapy are the primary treatment options for patients with TNBC

### THERATECHNOLOGIES' PLATFORM TECHNOLOGY

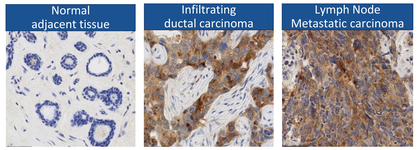
- New and flexible conjugation platform for personalized and targeted treatment of sortilin positive cancers
- Versatile conjugation strategies enabling different ratio of drug to peptide
- Proof of principle with conjugation of Theratechnologies peptide to anticancer drugs such as docetaxel (TH1902) for TNBC and doxorubicin (TH1904) for ovarian cancers


## Results

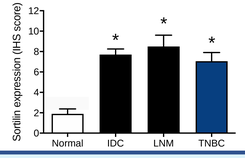
### HIGH SORTILIN EXPRESSION IN HUMAN BREAST CANCERS

#### Tissue Microarray (IHC)

- IHC showed high expression of sortilin in human breast cancers




- Highest expression levels were detected in lymph node metastases

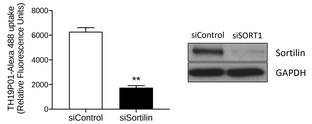



## Results (cont'd)

### SORTILIN-MEDIATED CHEMOTHERAPY INTERNALIZATION, PROLIFERATION, AND APOPTOSIS

#### A. Peptide-sortilin uptake (Sortilin gene silencing)

- Peptide uptake is inhibited upon sortilin siRNA




#### B. Anti-proliferation

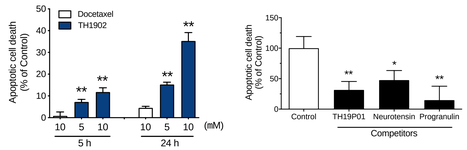
- TH1902 has a potent anti-proliferative activity

| | $IC_{50}$ (nM) | |
|---|---|---|
| Breast cancer cells | TH1902 | Docetaxel |
| **MDA-MB-231** | 0.19 ± 0.09 | 0.56 ± 0.19 |

#### C. TH1902 induces apoptosis of MDA-MB-231 cells

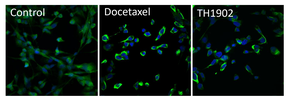
#### D. Reversal of TH1902 apoptosis by sortilin ligands

- Apoptosis induced by TH1902 was stronger than that of docetaxel and is reversed by sortilin ligands




#### E. TH1902 alters microtubules polymerization

- TH1902 alters MDA-MB-231 microtubules polymerization



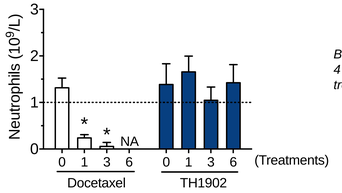
*Immunostaining of α-tubulin (green)*

## Results (cont'd)

### IN VIVO VALIDATION OF THERATECHNOLOGIES PLATFORM INCREASED EFFICACY WITH IMPROVED SAFETY

#### Absence of neutropenia

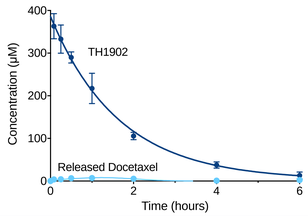
- **Absence of neutropenia** after 6 consecutive treatments of TH1902 at an equivalent dose of docetaxel (15 mg/kg/week)
- In contrast, 1 treatment with unconjugated Docetaxel strongly reduced neutrophil counts



*Blood was collected 4 days after each treatment*

#### Low docetaxel level in mouse plasma

- **High plasma concentration of TH1902** after IV bolus injection (50 mg/kg)
- **Very low concentration of docetaxel** released from TH1902 was measured in mouse plasma

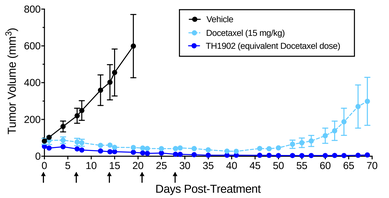



## Results (cont'd)

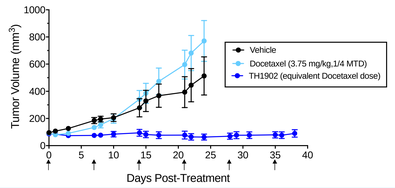
### STRONG INHIBITION OF TNBC TUMOR GROWTH (MDA-MB-231 S.C. XENOGRAFTS)

- **Better and sustained efficacy** with TH1902-treated mice at equivalent dose
- **Significant improvement of efficacy** with TH1902 when administered at lower dose
- **Higher cumulative injected dose** for TH1902 (up to 2-fold)

#### A. High dose (equivalent to Docetaxel MTD)




#### B. Low dose (equivalent to Docetaxel ¼ MTD)




**No significant side effects in TH1902-treated mice (no body weight loss and no neutropenia)**

## Conclusions

- A novel receptor-mediated chemotherapy, docetaxel-peptide conjugate
- Internalization mechanism in cancer cells requires Sortilin receptor
- Advantages of Theratechnologies Receptor-Mediated Chemotherapy (RMC):
  - Targets cancer overexpressing Sortilin receptor
  - Potential to increased therapeutic window of standard chemotherapy: improved tolerability (lower toxicity) and improved efficacy
  - Stronger inhibition of TNBC tumor growth compared to free drug
- Other breast cancer types expressing Sortilin could also potentially benefit from this novel therapeutic
- Validation of Theratechnologies novel platform for the development of personalized cancer therapies