AACR poster # 4493

Sudocetaxel Zendusortide (TH1902), a peptide-drug conjugate for the treatment of sortilin-positive (SORT1+) TNBC and HER2-positive breast cancers

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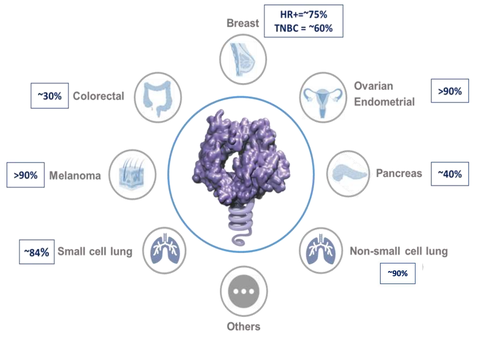
Introduction

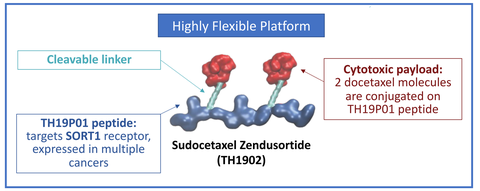
SORTILIN (SORT1) RECEPTOR IN CANCER

- Sortilin receptor is preferentially expressed in many cancers compared to healthy tissues, which makes it an attractive target for cancer drug development.
- SORT1 is a scavenger receptor involved in import-export of peptides into the cell via the endosomal/lysosomal pathway (cellular shuttle system).
- SORT1 is highly expressed in various tumor types (see AACR POSTER #3942 for more details):



SORT1⁺ TECHNOLOGY™ PLATFORM

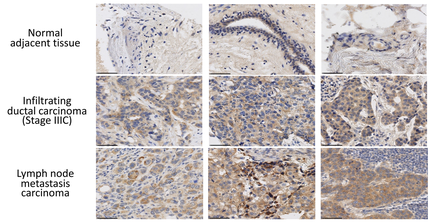
- **SORT1⁺ Technology™** is an innovative oncology platform consisting of novel peptides which target the SORT1 receptor.
- Targeting sortilin receptors with these peptide-drug conjugates (PDC's) leads to receptor-mediated internalization (endocytosis) of well-established anti-cancer agents (e.g., docetaxel, 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.
- Versatile and flexible conjugation strategies achieve different ratios of drug to peptide.



Results

HIGH SORTILIN EXPRESSION IN HUMAN TNBC AND HER2-POSITIVE BREAST CANCERS

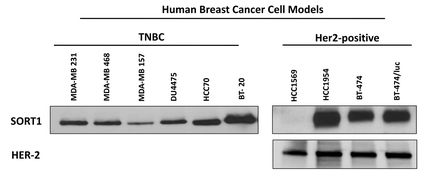
A. IHC of Sortilin Expression in Human Breast Cancer Tumors



- IHC shows a high levels of SORT1 expression in human breast cancers.

B. Western Blot

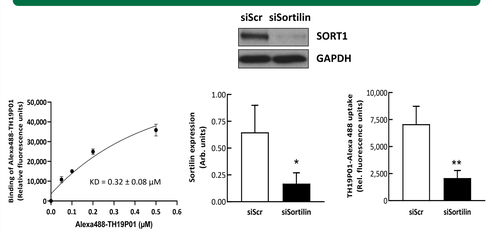
- Sortilin is highly expressed in several human TNBC and HER2-positive breast cancer cell lines.



Results (cont'd)

SORTILIN-MEDIATED INTERNALIZATION OF TH19P01 PEPTIDE

A. Binding and Uptake of fluorescent TH19P01 peptide

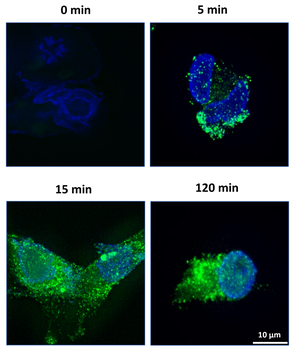


- TH19P01-Alexa488 binding is saturable, and its uptake is mediated by SORT1 in MDA-MB-231 cells.

Results (cont'd)

TH19P01 PEPTIDE INTERNALIZATION AND TRAFFICKING

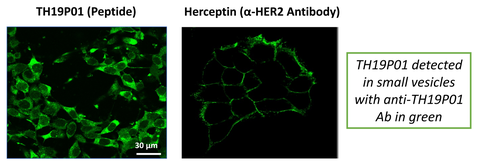
A. Rapid Internalization of TH19P01 in TNBC-Derived MDA-MB-231 Cells



Nuclei are stained in blue (DAPI)

TH19P01 detected in small vesicles with anti-TH19P01 Ab in green

B. Peptide vs Antibody Uptake in Cancer Cells



TH19P01 detected in small vesicles with anti-TH19P01 Ab in green

1h uptake in MDA-MB231 cells (TNBC)

1h uptake in BT-474 (HER2+ breast cancer) cells

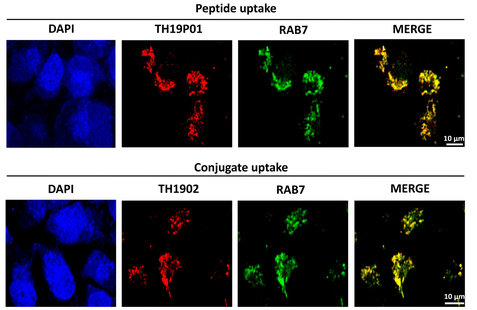
- *Source: Regina et al., Mol Cancer Ther; 14(1), 2015*

- Higher intracellular levels of TH19P01 peptide are observed in MDA-MB-231 cancer cells compared to Herceptin antibody.

Results (cont'd)

TH19P01 TRAFFICS THROUGH LYSOSOMES

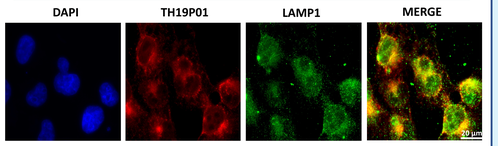
A. TH19P01 Peptide and TH1902 Conjugate Colocalize Within RAB7 Late Endosomes in MDA-MB-231 cells



- *Nuclei are stained in blue (DAPI)*
- *TH19P01/TH1902 detected in small vesicles with anti-TH19P01 Ab in red*
- *RAB7 classical late endosomal marker detected with anti-RAB7 Ab in green*

Cells were incubated for 60 min with TH19P01 or TH1902 at 37 °C, then washed and fixed before staining with anti-TH19P01 and anti-RAB7 Abs and suitable AlexaFluor-labelled secondary antibodies.

B. TH19P01 Colocalizes With LAMP1 Lysosomal Marker In MDA-MB-231 cells



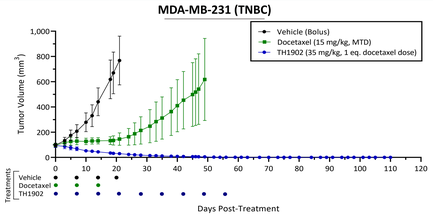
- *Nuclei are stained in blue (DAPI)*
- *TH19P01/TH1902 detected in small vesicles with anti-TH19P01 Ab in red*
- *LAMP1 lysosomal marker detected with anti-LAMP1 Ab in green*

Cells were incubated for 5 min with TH19P01 at 37 °C then washed before incubation in complete growth medium for 30 min.

Results (cont'd)

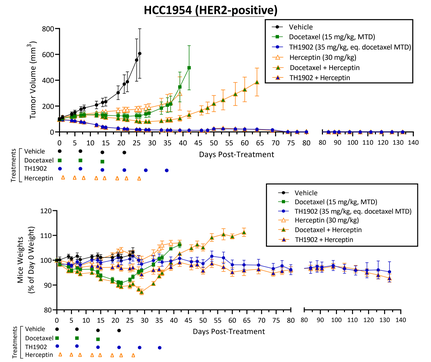
TH1902 LEADS TO COMPLETE REGRESSION OF TUMOR GROWTH IN BREAST CANCER XENOGRAFTS

A. Human TNBC (MDA-MB-231)



- 1 eq. dose: 9 cycles for TH1902 vs 3 for docetaxel
- Compared to docetaxel, TH1902 shows **better efficacy, sustained tumor regression, and higher tolerability** in MDA-MB-231 TNBC Xenograft Model.

B. HER2-Positive Breast Cancer (HCC1954)



- TH1902 alone or in combination with Herceptin leads to:
  1) Complete and sustained tumor regression,
  2) Body weight appears to be less affected than in docetaxel, or docetaxel/Herceptin-treated mice.

Conclusions

- SORT1⁺ Technology™ is an innovative and flexible platform. It relies on the use of a novel peptide (TH19P01) that can be conjugated to numerous well-characterized anticancer drugs.
- SORT1 is expressed in 77% of HER2-positive breast cancers (Roselli *et al.*, 2015).
- TH19P01 has been designed to interact with and be transported by the scavenger receptor sortilin (SORT1) which functions are involved in protein internalization, sorting, and trafficking.
- Conjugation of TH19P01 has the potential to limit the systemic toxicity of anticancer drugs.
- Once SORT1 gene expression is reduced by specific siRNA, TH19P01-Alexa488 peptide internalization is drastically decreased in MDA-MB-231 cells supporting the SORT1-mediated internalization process.
- Rapid uptake in cancer cells is observed for TH19P01 peptide, which accumulates in intracellular compartments.
- TH19P01 peptide colocalizes with RAB7 (late endosomal marker) and LAMP1 (lysosomal marker). This suggests its ability to recognize SORT1 and to be internalized through endocytic pathways leading to lysosomes.
- TH1902 (at equivalent docetaxel MTD doses) demonstrated a better anticancer efficacy over docetaxel, complete tumor regression, and higher tolerability in TNBC and HER2-positive breast cancer xenograft models.
- HCC1954 breast cancer model (HER2-positive), known to be less sensitive to Herceptin, showed a very good response to TH1902 as a single agent even when compared to combined Herceptin/docetaxel treatment.

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