AACR abstract # 2071

Pre-clinical evidence for new camptothecin-peptide conjugates in the treatment of sortilin-positive colorectal cancers

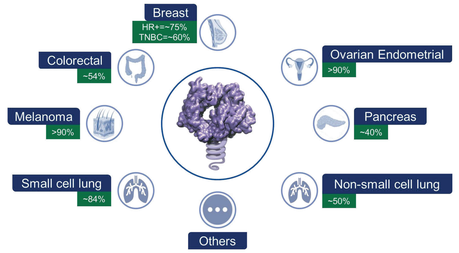
Sanjoy Kumar Das[1], Jean-Christophe Currie[1], Michel Demeule[1], Cyndia Charfi[1], Alain Zgheib[2], Amit Nayyar[1], Anh Minh Thao Nguyen[1], Bogdan Alexandru Danalache[2], Richard Béliveau[2], Christian Marsolais[1], Borhane Annabi[2]

[1]Theratechnologies Inc., Montreal, QC, Canada and [2]Université du Québec à Montréal, Montreal, QC, Canada

Introduction

SORTILIN (SORT1) RECEPTOR IN CANCER

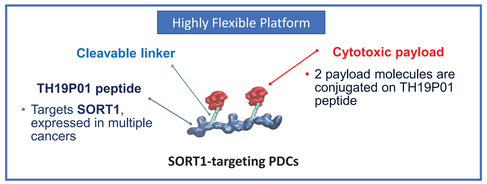
- Sortilin (SORT1) is highly expressed in many cancers compared to healthy tissues, which makes it an attractive target for cancer drug development.
- SORT1 is a transmembrane scavenger receptor involved in import-export of peptides into the cell via the endosomal/lysosomal pathway (cellular shuttle system).
- SORT1 plays multiple roles in cancer; associated with progression, invasion, and aggressive disease [1,2].
- SORT1 is an ideal candidate for internalization of peptide-drug conjugates (PDCs).
- Known sortilin expression in various tumor types [3,4]:



- SORT1 is expressed in about 54% of colorectal cancers (CRC).

SORT1⁺ TECHNOLOGY™ PLATFORM

- **SORT1⁺ Technology™** is an innovative oncology platform consisting of novel peptides that target the SORT1 receptor.
- Targeting SORT1 with platform-derived PDCs leads to receptor-mediated internalization (endocytosis) of anti-cancer agents.
- Sudocetaxel Zendosortide (TH1902) is currently under investigation in a phase 1 clinical trial (NCT04706962).
- Once inside cancer cells, active drug is released from the peptide and exerts its cytotoxic effect directly on the cancer cell.
- The platform enables versatile and flexible conjugation strategies with different payloads and linkers.



Results

In vitro characterization

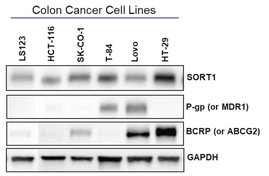
A. Peptide-drug conjugates description

Peptide-Drug Conjugate (PDC) Components

Compound ID	Payload	Linker	Linker Sensitivity
TH2101	SN-38	Gly-Osu	Esterase (pH)
TH2205	SN-38	Gly-PEG$_3$	Esterase (pH)
TH2310	SN-38	Lys-Osu	Esterase (pH)
TH2303	Exatecan	Gly-Osu	Esterase (pH)
TH1902	Docetaxel	Osu	Esterase (pH)

[1] All conjugates were synthesized using the TH19P01 peptide

B. Sortilin (SORT1) expression in CRC cell lines



- SORT1 is expressed in several human CRC cell lines.
- Differential expression of efflux pumps P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP).
- PDCs' *in vivo* anti-cancer efficacies were investigated in CRC xenograft models HT-29 (BCRP-high) and HCT-116 (BCRP-low).

C. Inhibition of CRC cell proliferation

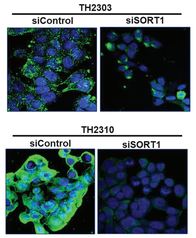
Cell proliferation inhibition in CRC cell lines (IC_{50} values, nM)

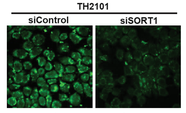
Drug	HT-29	HCT-116	LoVo
Irinotecan[2]	5079 (6)	NA	4871 (5)
SN-38[1]	65.6 (4)	17.9 (3)	51.1 (7)
TH2101[1]	88.9 (3)	NA	63.3 (4)
TH2205[1]	76.5 (1)	NA	63.8 (1)
TH2310[1]	124.9 (2)	32.1 (2)	88.7 (2)
Exatecan[2]	3.5 (5)	0.86 (2)	3.0 (4)
TH2303[2]	9.2 (1)	1.7 (1)	3.8 (1)

[1]72h incubation time, [2]96h incubation time, NA: not available, n number in parenthesis.

- Greater anti-proliferative activities for Exatecan- and SN-38-conjugates compared to Irinotecan.

D. SORT1-mediated internalization





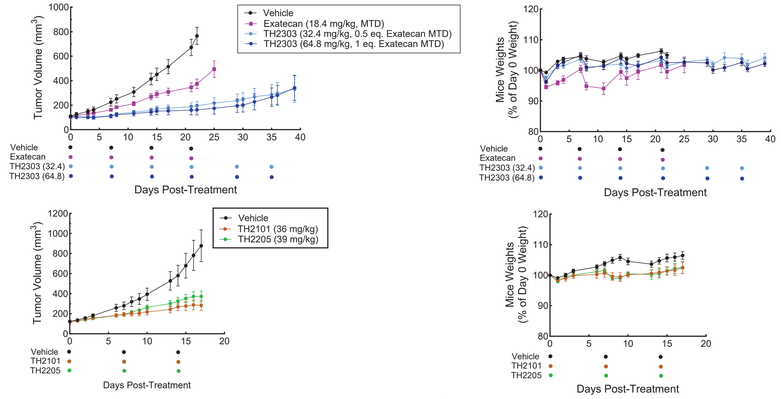
Green: Fluorescence from TH2101

Blue: Nuclei staining using DAPI
Green: TH2303/TH2310 detection using anti-TH19P01 antibody

- SORT1 gene silencing inhibits PDC internalization in cancer cells.
- Suggests that these PDCs enter cancer cells via a SORT1-mediated internalization process.

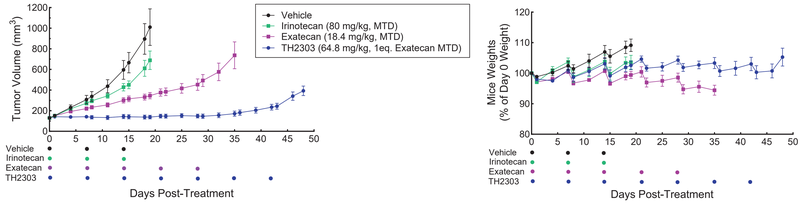
Increased anti-cancer activity in xenograft models for camptothecin-conjugates

A. HT-29 CRC xenograft model (BCRP-high)



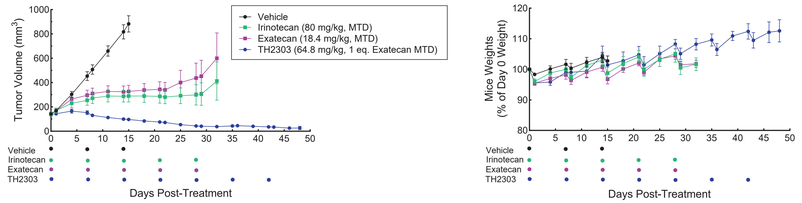
- Increased tumor growth inhibitions and tolerability for TH2303 (exatecan-conjugate) and TH2101 (SN-38-conjugate) in the HT-29 BCRP-high CRC model compared to exatecan or irinotecan unconjugated drugs.

B. HCT-116 CRC xenograft model (BCRP-low)



- Increased tumor growth inhibitions and tolerability for TH2303 (exatecan-conjugate) in the HCT-116 BCRP-low CRC model compared to exatecan or irinotecan unconjugated drugs.

C. Triple-negative breast cancer (TNBC) xenograft model (MDA-MB-231)

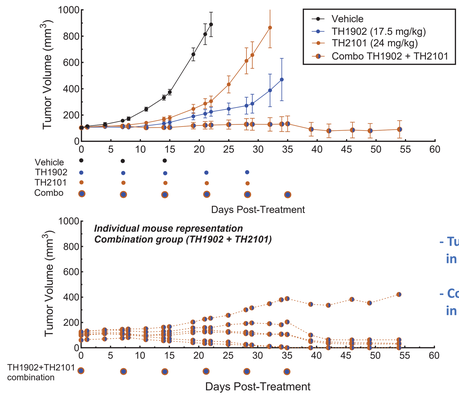


- Increased tumor growth inhibitions and tolerability for TH2303 (exatecan-conjugate) in the MDA-MB-231 TNBC model compared to exatecan or irinotecan unconjugated drugs.

Results (cont'd)

Increased anti-cancer activity in xenograft models for camptothecin-conjugates

D. Combination of two SORT1-targeting PDCs in HT-29 xenograft model (BCRP-high)



- Tumor regression >50% in 5 of 6 mice (83%)
- Complete tumor regression in 2 of 6 mice (33%)

- Reduced doses of TH1902 (docetaxel-conjugate) or TH2101 (SN-38-conjugate) were administered alone or in combination.
- Combination of two SORT1-targeting PDCs led to:
  1) Increased tumor growth inhibition and some complete responses.
  2) Good tolerability according to mice body weight (data not shown).

Conclusions

- SORT1⁺ Technology™ is a versatile and flexible platform. It relies on the use of a novel peptide (TH19P01) that can be conjugated to numerous well-characterized anti-cancer drugs.
- TH19P01 has been designed to interact with and be transported by the scavenger receptor sortilin (SORT1), which is involved in protein internalization, sorting and trafficking.
- Based on body weight, TH2101 (SN-38)- and TH2303 (exatecan)-conjugates were well tolerated.
- Following SORT1 gene silencing, camptothecin-conjugate uptake is drastically decreased in HT-29 cells, supporting the SORT1-mediated internalization process.
- TH2101 and TH2303 demonstrated greater anti-cancer efficacy compared to irinotecan and exatecan in two CRC xenograft models, independently of BCRP efflux pump expression levels.
- In addition, significant tumor regression (over 80%) was observed following administration of TH2302 in the MDA-MB-231 TNBC xenograft model.
- Notably, in the HT-29 xenograft model, which is known for resistance to multiple cytotoxic drugs, the combination of two PDCs (TH2101 and TH1902) led to significant tumor regression.

[1] Ghaemimanesh F, et al., J Cell Physiol. 2021;236(9):6271-6281. [2] Roselli S, et al., Oncotarget. 2015;6(12):10473-10486. [3] Roy G, et al., Eur J Cancer. 2022;174S1:S117 (abstr 328). [4] Roy G, et al., Cancer Res. 2024; 83(Supp7):3942.

Acknowledgements. This study was funded by a SynergiQc grant from the CQDM biopharmaceutical consortium and the Fondation Cancer du Sein du Québec, and by the Canadian Cancer Society.