Anti-cancer activity of TH-1011, a novel Hypoxia-activated prodrug, is related to tumor vessel disruption

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THRESHOLD PHARMACEUTICALS

Abstract # 1442

Vascular disrupting agents (VDA) could shut down the neo- and existing vasculature in tumors, extend the hypoxic zone and lead to increased necrosis of tumor tissue. TH-1011 is a hypoxia activated prodrug of the parent toxin, TH-482, which is a VDA and microtubule inhibitor. In the present study, we evaluated the efficacy of TH-1011 in combination with irinotecan (CPT-11) in the HT29 colorectal xenograft model and investigated the possible anti-cancer mechanisms focusing on vessel perfusion.

$3x10^6$ HT29 cells were implanted subcutaneously. In the efficacy studies, mice were treated with Vehicle, CPT-11 alone (50mg/Kg, Q7D x3, ip), TH-1011 alone (Mono, 15mg/Kg, Q2Dx3/wk x 2wks), and combination of the two compounds (Combo). Daily dosing of TH-1011 for 5days/wk, total 2 wks, ip was also tested. In vessel perfusion studies, time course and dose response experiments were performed. Combretastatin A-4 phosphate (CA4P), a well known VDA, served as a positive control.

TH-1011 every other day treatment, significantly inhibited and delayed the tumor growth. 23 days after the treatment, tumor growth inhibitions (TGIs) were 51.3%, 42.7% and 81.4% in Mono, CPT-11 alone and Combo groups respectively. There was no body weight loss in any group during the study period. Daily dosing of TH-1011 resulted in less efficacy than every-other-day dosing, TGIs were only 13.5% and 54.9% in Mono and Combo groups respectively. Tumor vessel perfusion status was demonstrated by Hoechst 33342 staining. Untreated tumor samples were well perfused throughout the tumor area in both time course and dose response studies. Time course study shows 1 hour after TH-1011 treatment, vessels were almost completely shut down, and after 24 hours partial closure of vessels remained evident. In dose response study, 5mg/Kg and 1.5mg/Kg TH-1011 could induce partial shutdown of the vessels. These results demonstrate that TH-1011 alone is effective in the HT29 colorectal xenograft model, and when combined with CPT-11, the anti-tumor activity of TH-1011 is greatly enhanced. The tumor inhibition mechanism may be related to the tumor vessel disruption. The observed reduced efficacy of TH-1011 daily dosing, compared to less frequent dosing, under test conditions, could be due to the partial vessel shutdown in the tumor within 24 hours.

Introduction

- ♦ Tumor vasculature is critical for survival and the continuous growth of tumors.
- ♦ Vascular disrupting agents could shut down the neo- and existing vasculature in tumors, extend the hypoxic zone and lead to increased necrosis of tumor tissue.
- ♦ TH-1011 is a hypoxia activated prodrug of the parent toxin, TH-482, which is a VDA and microtubule inhibitor.
- ♦ CPT-11, a topoisomerase I inhibitor, has established activity against advanced colorectal cancer
- ♦ Combretastatin A4 phosphate (CA4P) exerts its anti-angiogenic effect by selectively targeting endothelial cells

Methods

♦ Efficacy Studies:

Tumor

- HT29 colorectal carcinoma, s.c implantation with $3x10^6$ cells
- Randomize when average size of tumor was $150mm^3$ on Day 1 and start treatment

Groups (N=10/group)

- Vehicle
- CPT-11 alone (50mg/Kg, ip, Q7D x3)
- TH-1011 mono (15mg/Kg, ip, every-other-day dosing or daily dosing)
- TH-1011 + CPT-11

♦ Vessel Studies

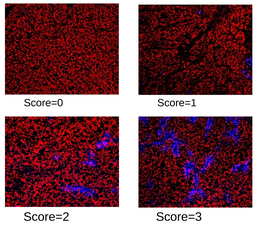
- Tumor size $300\text{-}500mm^3$
- Hoechst 33342 was iv. injected 1min before euthanization

Time Course Study

- TH-1011 15mg/Kg, ip
- Xenograft tumor was harvested **1, 4, 8** and **24**hrs after treatment
- Pimonidazole for labeling hypoxia cells.

Dose Response Study

- Animals were ip treated with TH-1011 at **1.5, 5** and **15**mg/Kg
- Xenograft tumor was harvested 1hr after treatment
- Semi-quantitative methods



Shut-down: Score=0
Partial Shut-down: Score=1 or 2
Non-Shut-down: Score=3

Results

Efficacy Study

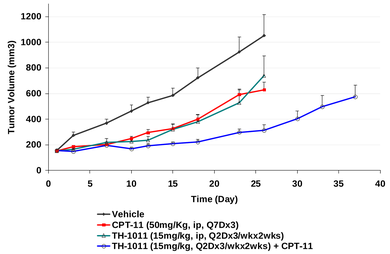


Fig 1. Anti-tumor activity assessment from **every-other-day dosing** of TH-1011 in HT-29 model.

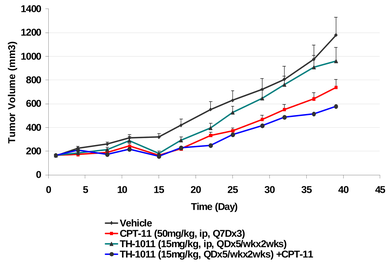


Fig 2. Anti-tumor activity assessment from **daily dosing** of TH-1011 in HT-29 model.

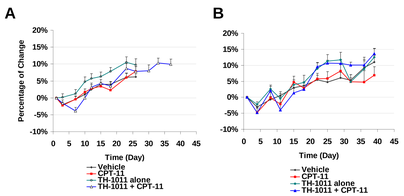


Fig 3. Body weight change in TH-1011 efficacy studies. A, Every-other-day dosing; B, daily dosing.

Vessel studies

Time Course

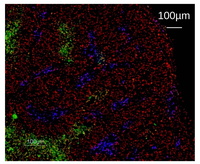


Fig 4. Fluorescence microscope of a control untreated tumor. Vessel perfusion (blue, Hoechst 33342), hypoxia (Green, Pimonidazole) and tumor morphology (red, Propidium Iodide).

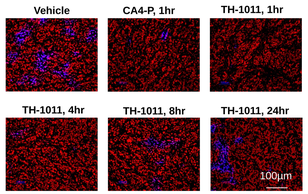


Fig 5. Time course study: effect of TH-1011 on HT29 tumor vessels. TH-1011, 15mg/Kg, ip. CA4-P 100mg/Kg, ip.

Dose Response

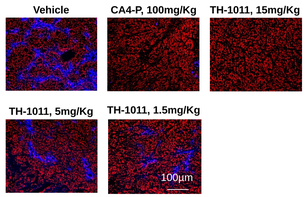


Fig 6. Dose response study: effect of TH-1011 on HT29 tumor vessels. Tumors were harvested 1hour after treatment.

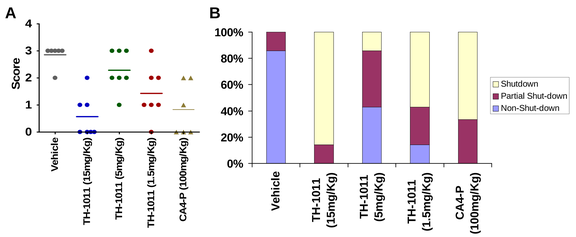


Fig 7. Semi-quantitative analysis showing blood vessel perfusion after treatment with TH-1011 or CA4-P. A, individual tumor score; B, tumor vessel shut-down status.

Summary

Efficacy Studies

- ♦ TH-1011 every-other-day treatment significant inhibited and delayed the tumor growth.
- ♦ Daily dosing of TH-1011 exhibited less efficacy than every-other-day dosing.
- ♦ Every-other-day dosing of TH-1011 did not show toxicity as judged by gross examination and body weight change. However, daily dosing resulted in animal death.

Treatment		Efficacy		Toxicity
TH-1011 (15mg/Kg, ip)	Combination Therapy	TGI (%)	TGD_{500} (day)	Animal death
Every Other Day Q2D x3/wk x 2wks	-	51.3	11	0/10
	+ CPT-11	81.4 (42.7)	22 (10)	0/10
Daily QD x5/wk x 2wks	-	8.2	4	1/10
	+ CPT-11	56.9 (41)	15 (11)	1/10

Table 1. Summary of every-other-day dosing and daily dosing of TH-1011 on anti-tumor activity and toxicity.

TGI: Tumor growth inhibition on the last day all control animals were alive

TGD_{500}: Tumor growth delay, extra days for the treated tumor to reach $500mm^3$ as compared to the vehicle group.

The number in ( ) are the effect by chemotherapy, CPT-11 alone.

Vessel Studies

- ♦ Hypoxia exists in the HT29 human colon cancer xenograft tumor.
- ♦ TH-1011, 15mg/Kg, ip almost completely shut down the vessel after 1hr but still remained partially open after 24hrs.
- ♦ 5mg/Kg and 1.5mg/Kg of TH-1011 resulted in vessel partial shutdown after 1hr treatment. There was no clear dose response.

Conclusions

- ♦ **TH-1011 alone is effective in the HT29 colorectal xenograft model. Anti-tumor activity of TH-1011 is greatly enhanced when combined with CPT-11.**
- ♦ **Tumor inhibition mechanism may be related to the tumor vessel disruption.**
- ♦ **The observed reduced efficacy of TH-1011 daily dosing, compared to less frequent dosing, under test conditions, could be due to the partial vessel shutdown in the tumor within 24 hours.**