Anti-cancer activity of hypoxia activated prodrug, TH-302 in H460 non-small cell lung carcinoma metastatic model

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THRESHOLD PHARMACEUTICALS

Abstract

The hypoxia activated phosphoramidate prodrug, TH-302, has showed anti-tumor activity in a variety of in vivo models. As a DNA alkylating agent, TH-302 is selectively activated in hypoxic conditions and currently in Phase I clinical evaluation. In the present study, we investigated the efficacy of TH-302 in combination with docetaxel in a non-small cell lung carcinoma (NSCLC) metastatic model based on the inoculation of tumor cells into the pleural space of nude mice.

$1x10^6$ H460 human NSCLC cells were implanted through the chest wall into left pleural space of nude mice in a volume of 100µl. 7, 14 and 21 days after implantation, necropsy and histology was performed to characterize the model. In the efficacy studies, mice were randomized into 4 groups (N=12) 7 days after implantation: Vehicle, docetaxel alone (10mg/Kg, Q7D x2, iv), TH-302 alone (50mg/Kg, QDx5/wk x 2wks, ip), and combination of the two compounds. Animals were monitored daily until endpoints were reached, which included spontaneous death or body weight loss more than 20% or cachexia produced severe morbidity. Karplan-Meier survival analysis was performed. The antitumor activity was evaluated as T/C%= mean survival time (MST) of treated group/ MST of vehicle group x 100. Results were also expressed as the percentage of Increased Lifespan (ILS= T/C of treated group-100).

A few tumor nodules could be observed in the lung at Day 7. Tumor cells started to invade mediastinum, heart and the whole thoracic cavity from Day 14. Hypoxia was detected in the metastatic tumors by pimonidazole staining. Around day 21, the metastatic tumor progression in the pleural cavity was very aggressive. Distant metastatic sites were seen in lymph nodes and ovaries. In the efficacy studies, during the 90-day observation, MST was 24, 35, 43 and 56 days in Vehicle, docetaxel alone, TH-302 alone and combination groups, respectively. Docetaxel alone or TH302 alone showed certain antitumor activity, T/C was 146% and 177%, respectively. However, the anti-cancer effect was significantly increased in the combination group, inducing a maximal ILS of 133% (T/C=233%, p<0.01). Average time to 50% survival was 21, 26, 34 and 35 days in Vehicle, docetaxel alone, TH-302 alone and combination groups, respectively. TH-302 in combination with docetaxel showed significantly survival time increased comparing with vehicle (p<0.05). There was no statistical difference between docetaxel alone group and vehicle on average time to 50% survival.

Introduction

- ♦ TH-302 is a hypoxia activated prodrug comprised of a nitroimidazole hypoxic trigger conjugated to a cytotoxic moiety (bromo-isophosphoramide mustard; Br-IPM) and undergoes activation in severe (<1% O_2) hypoxia.
- ♦ TH-302 has showed anti-tumor activity in a variety of in vivo models including ectopic, orthotopic as well as metastatic models.
- ♦ Intrapleural injection of H460, a non-small cell lung cancer line, shows 100% incidence of metastasis and the growth patterns of it are similar to that encountered in lung cancer patients. Moreover, this model mimics, in a few weeks, an advanced stage of the human disease.
- ♦ Degree of hypoxia observed in the disseminated metastasis in the model is dependent on the tumor size. Micrometastatic tumors(diameter<1mm) exhibit severe hypoxia.

Methods

Establishment of Metastasis Model

(Kraus-Berthier *et al.* Ref. 1)

Cells

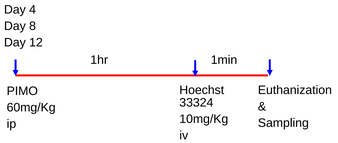
- H460, non-small cell lung carcinoma, $1x10^6$ cells were implanted through the chest wall into the left pleural space of nude mice
- Implantation day was Day 0.

Macroscopic Study

- 4 mice/group
- Necropsy was performed on Day 7, 14 and 21.

Microscopic Study

- 3-4 mice/group
- Lung and mediastinal organs were collected on Day 4, 8 and 12.
- Immunofluorescence and H&E staining were performed.
- Pimonidazole (PIMO) employed as hypoxia marker
- Hoechst 33324 used to mark tumor vasculature and perfusion.



In Vivo Antitumor Activity

Tumor Model

- H460, intrapleural implantation with $1x10^6$ cells
- Randomize on Day 7 and started treatment

Groups (N=12/group)

- Vehicle
- Docetaxel alone (10mg/Kg, iv, Q7D x2)
- TH-302 alone (50mg/Kg, ip, QDx5/wk x 2wks)
- TH-302+ Docetaxel

Evaluation

- Karplan-Meier survival analysis
- Antitumor activity T/C%= mean survival time (MST) of treated group/ MST of Vehicle group x 100
- Percentage of Increased Lifespan (ILS= T/C of treated group-100)
- Log-rank test for comparing the treated groups to the vehicle group.

Results

Metastasis and Hypoxia

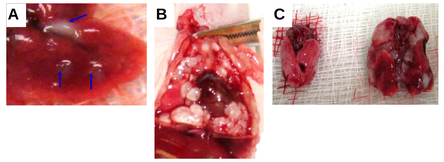


Fig 1. Macroscopic observation on the progression of H460 intrapleural metastasis. A, Tumor nodules (arrows) on Day 7; B, Day 14; C, Day 21. Left, normal lung and right, lung with aggressive metastasis.

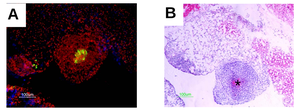


Fig 3. Representative images from H460 intrapleural metastasis tissue on Day 4. A, overlay image [PIMO (green), Hoechst 33342 (blue) and Propidium Iodide (red)] and B, H&E staining on the consecutive frozen sections. *, necrosis

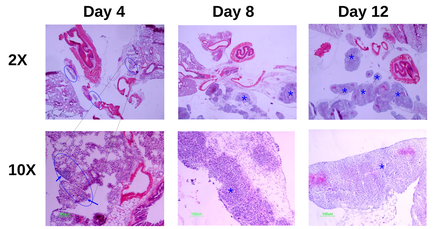


Fig 2. Representative microscopic findings on the progression of H460 intrapleural metastasis by H& E staining. *, metastatic tumor.

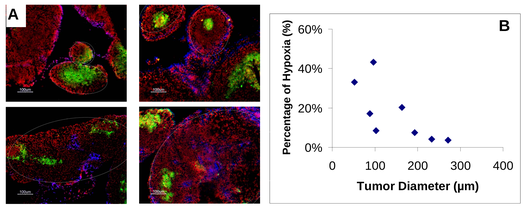


Fig 4. Immunofluorescence overlay images showing hypoxia in the tumors with different size (A) and relationship between tumor size and % hypoxia by scatterplot (B).

In Vivo Efficacy of TH302

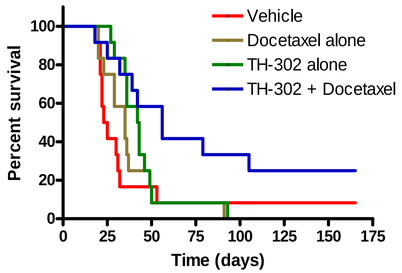


Fig 5. Antitumor activity of TH-302 in combination with Docetaxel in the H460 intrapleural metastasis model.

	MST (days)	T/C%	ILS %
Vehicle	24		
Docetaxel alone	35	146	46
TH-302 alone	43	177	77
TH302 + Docetaxel	56	233	133

Table 1. Antitumor activity of TH302 in combination with Docetaxel in the H460 intrapleural metastasis model. The survival curve of each treated group was compared with that of Vehicle by the log-rank test. MST, median survival time; T/C%= MST of treated group/ MST of Vehicle group x 100; ILS, increased lifespan=T/C of treated group-100; *, p<0.05.

References

1. Kraus-Berthier *et al. Clinical Cancer Res* 2000: **6**: 297-304
2. Duan *et al. J Med Chem* 2008: **51**: 2412-2420
3. Hohla *et al. PNAS* 2006: **103**: 14513-14518
4. Li *et al. Cancer Res* 2007: **7**: 7646-7653

Summary

H460 Intrapleural Metastasis Model

- ♦ Following intrapleural implantation of H460 cells (*Fig. 1 and 2*),
- Day 4: tumor cells or tumor cell clusters were attached to the surface and the edge of the lung as well as other mediastinal organs.
- Day 8: the metastasis tumors spread within the lung.
- Day 12: metastasis tumors gradually invaded the whole thoracic cavity.
- Day 21: metastasis was very aggressive, about 50% animals dead.

Hypoxia Characterization of Metastasis

- ♦ Hypoxia could be detected in the metastasis as early as Day 4 (*Fig. 3*).
- ♦ Micromets (diameter <1 mm) were dramatically hypoxic and poorly perfused; the smallest mets (several hundred microns diameter) were avascular. With mets size increasing, tumor exhibited more vasculature and less hypoxia (*Fig. 4)*.
- ♦ Hypoxic area in the metastasis varied from 5% to almost 50% depending on the tumor size (*Fig. 4B)*.

Antitumor Activity of TH-302

- ♦ TH-302 alone showed longer MST (Median Survival Time) as well as ILS (Increased Lifespan) in this model when comparing with docetaxel alone (*Fig. 5 and Table 1)*.
- ♦ With TH302 in combination with docetaxel, the antitumor activity was greatly enhanced, demonstrated as significantly increased T/C% and ILS% (*Fig. 5 and Table 1)*.

Conclusions

- ♦ An intrapleural metastasis model is established by pleural implantation of H460, a non-small cell lung carcinoma cell line in nude mice.
- ♦ TH-302 in combination with docetaxel shows significant antitumor activity. Therefore, this drug combination may be particularly useful for treatment of metastatic disease associated with non-small cell lung cancer
- ♦ The severe hypoxia observed at early stage in this model may be related to the high efficacy of TH-302.