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# **Discovery and Preclinical Development of TH-302: a hypoxia-activated cytotoxic prodrug**

ASCO Annual Meeting - June 2007

# TH-302

## Discovery and Preclinical Development

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- ▶ Introduction
    - Targeting tumor hypoxia
  
  - ▶ Drug Discovery Strategy
    - Chemistry
    - Biology
  
  - ▶ Preclinical Data
    - *in vitro*
    - *in vivo*
  
  - ▶ Conclusions
    - Clinical status
-

# Targeting the Tumor Microenvironment

## Hypoxia as a central feature

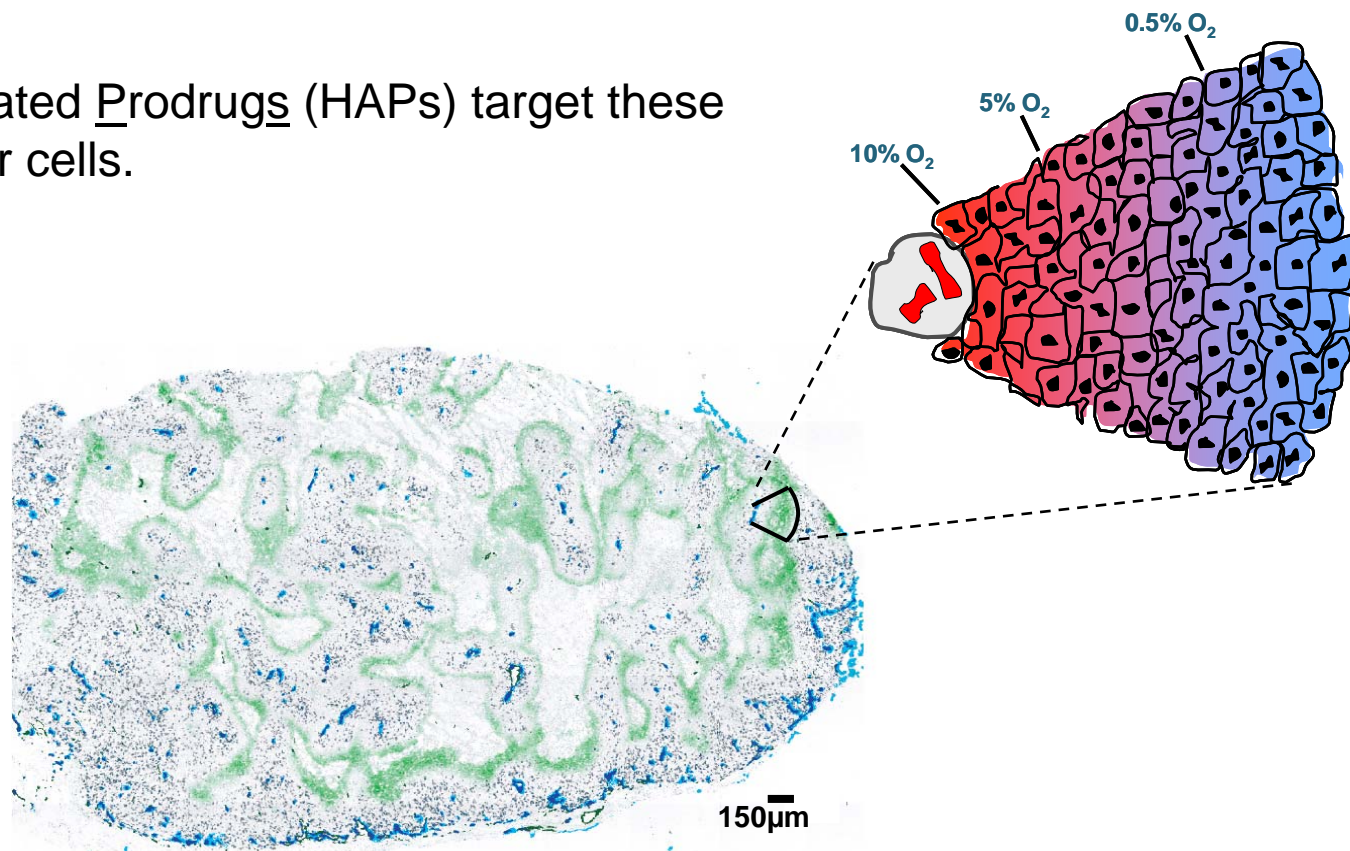


- ▶ There is greater appreciation of the role of the tumor microenvironment in cancer progression
- ▶ Differences between tumor microenvironment and surrounding normal tissue can be exploited in the design of new anticancer drugs
- ▶ Microenvironmental hallmarks of solid tumors:
  - Low oxygen tension (**hypoxia**)
  - Glycolytic phenotype
  - Low extracellular pH
  - High interstitial fluid pressure
  - Altered tumor vasculature
  - Tumor associated macrophages (TAMs)

# Targeting tumor hypoxia

## Hypoxia-Activated Prodrugs (HAPs)

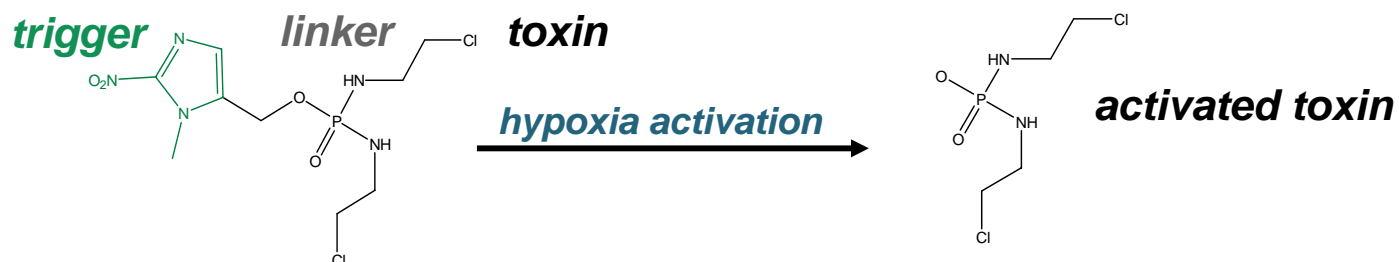
- ▶ Hypoxic cancer cells resist conventional chemotherapy and radiation.
- ▶ Hypoxia-Activated Prodrugs (HAPs) target these resistant tumor cells.



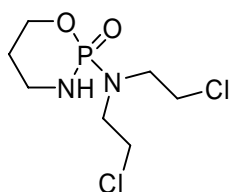
A. Minchinton, University of British Columbia.

# Chemistry Strategy

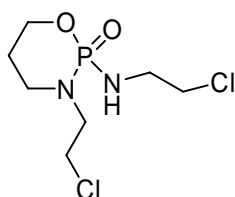
## Bioreductive or Hypoxia-Activated Prodrugs (HAPs)



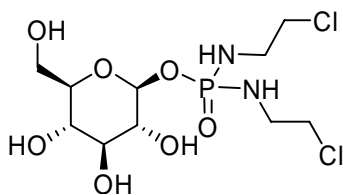
### Bis-alkylator Drugs



Cyclophosphamide

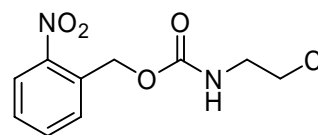


Ifosfamide

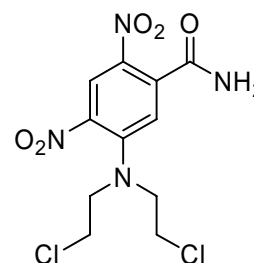


Glufosfamide

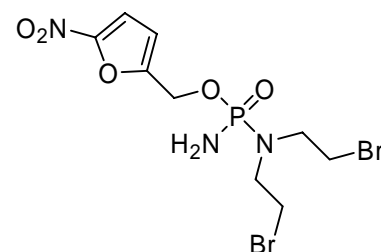
### Triggering strategies



Teicher and Sartorelli, 1980



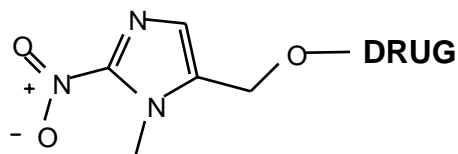
Denny and Wilson, 1986



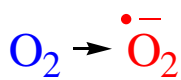
Borch *et al.*, 1986

# Chemistry Strategy

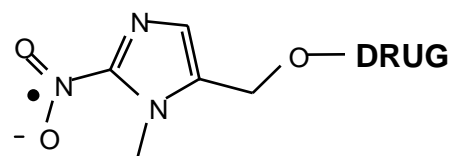
## Bioreductive or Hypoxia-Activated Prodrugs (HAPs)



*NADPH cytochrome  
P450 reductase*

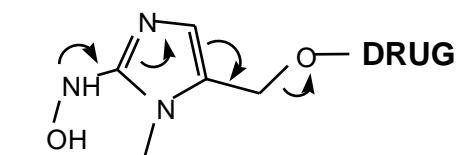


Non-toxic prodrug reduced to radical substrate for back-oxidation to the original compound with  $\text{O}_2$

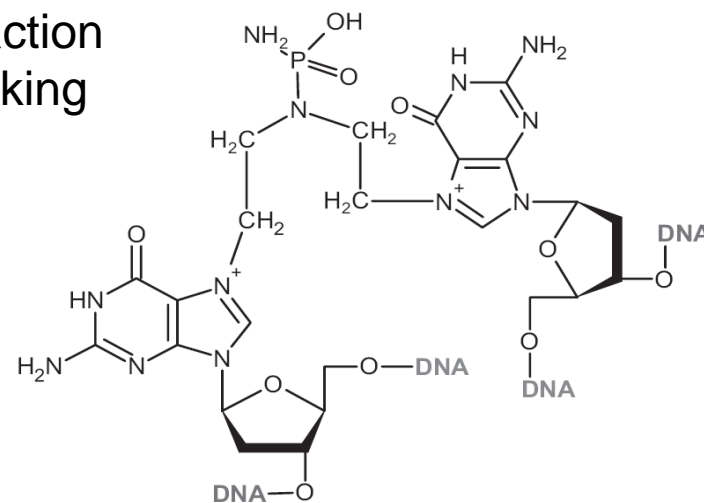
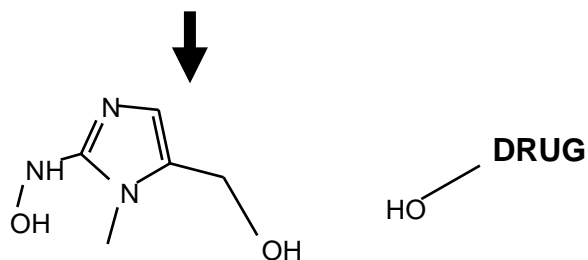


Absence of oxygen and subsequent reduction releases the toxin

*further  
reduction*

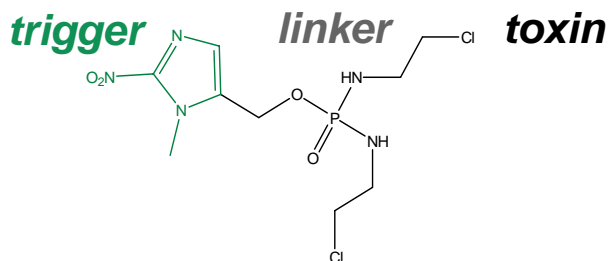


Mechanism-of-action is DNA cross-linking

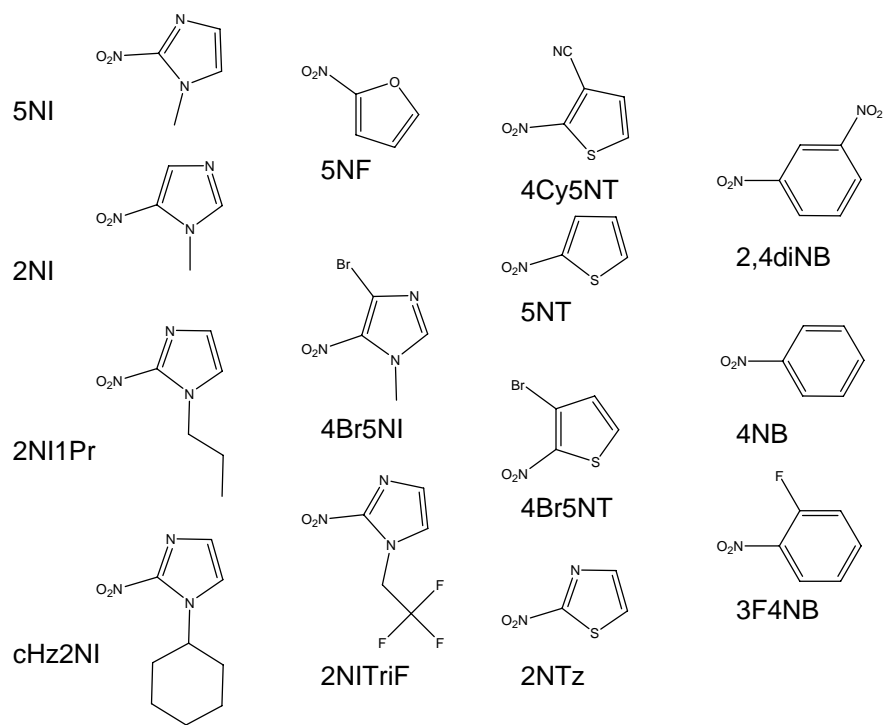


# Hypoxia-Activated Prodrugs

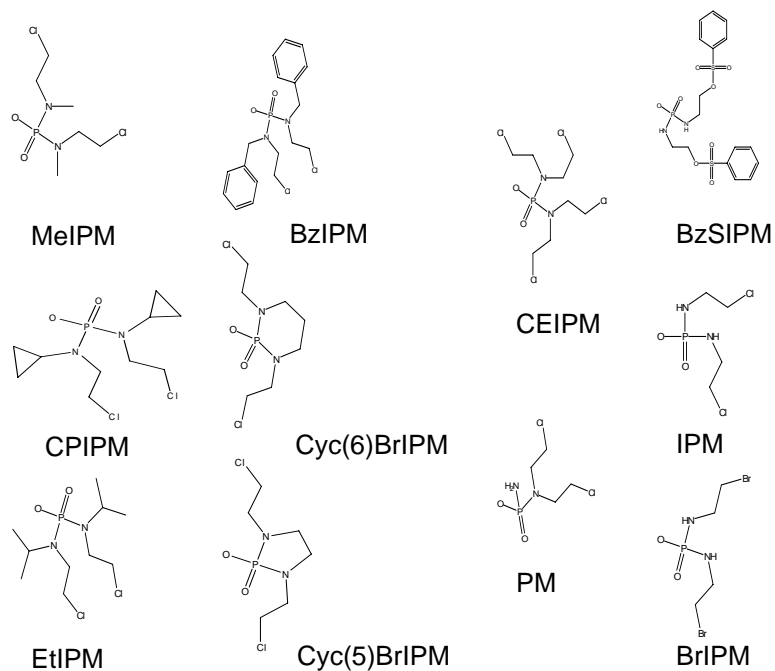
A modular combinatorial chemistry strategy



## Triggers

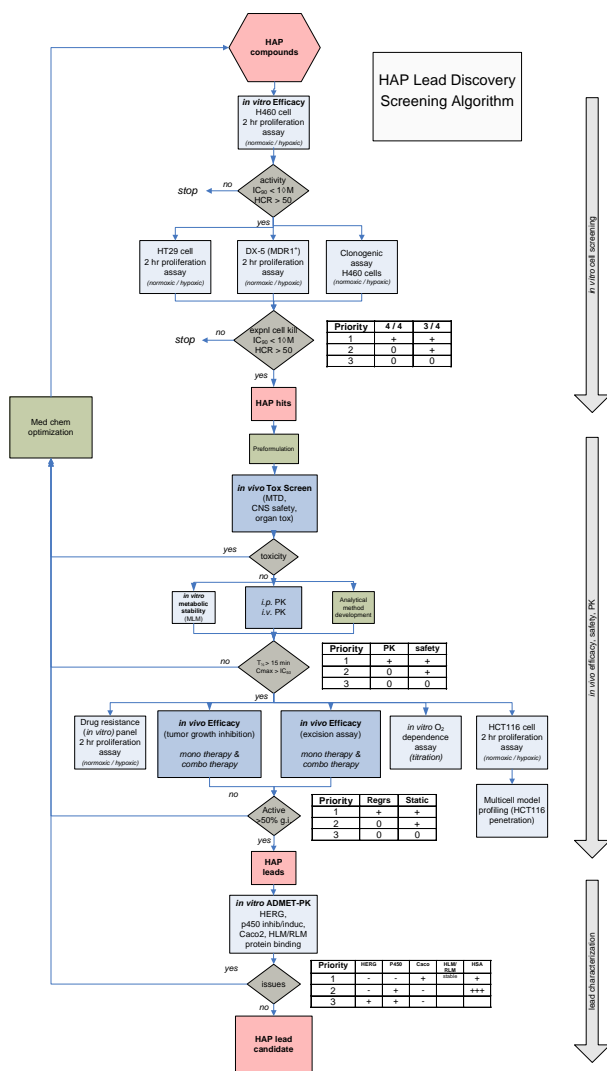


## Toxins



# Hypoxia-Activated Prodrug Discovery

The corresponding biological screening algorithm



*in vitro* efficacy

*in vitro* ADME

*in vivo* PK

*in vivo* efficacy

tox

► Series of activity gates the compounds must transit

► The gates question compound attributes

- Positive attributes
  - *e.g.* *in vivo* efficacy
  - adequate PK
- Negative attributes
  - *e.g.* renal toxicity

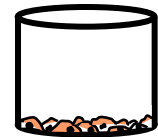
► Criteria are set for the compound to progress through a gate



# TH-302 *in vitro* cytotoxicity profiling

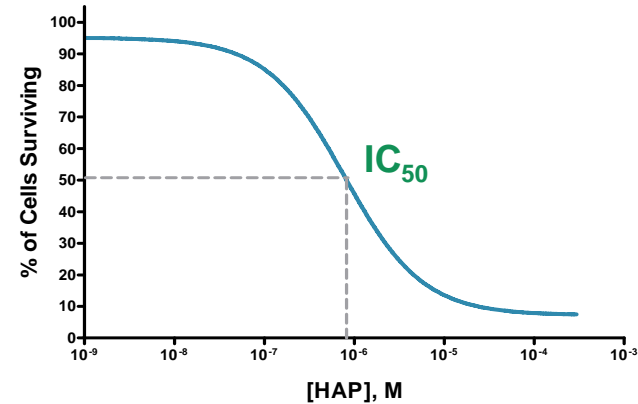
Cell viability assay and clonogenic survival assay

## Cell viability assay



+/- Drug  
+/- Hypoxia

Grow for  
3-4 days

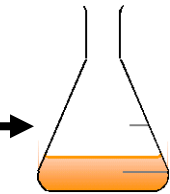


Determine cell viability

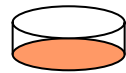
## Clonogenic survival assay



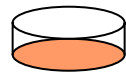
+/- Drug  
+/- Hypoxia



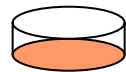
Dissociate  
Cells



10<sup>2</sup>



10<sup>3</sup>



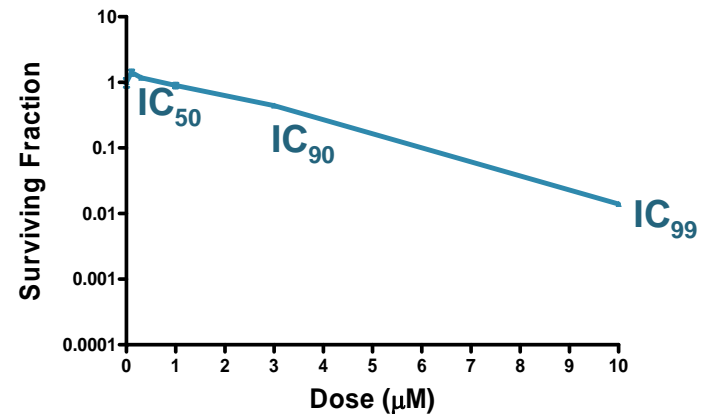
10<sup>4</sup>



10<sup>5</sup>

Plate  
Serial Dilution

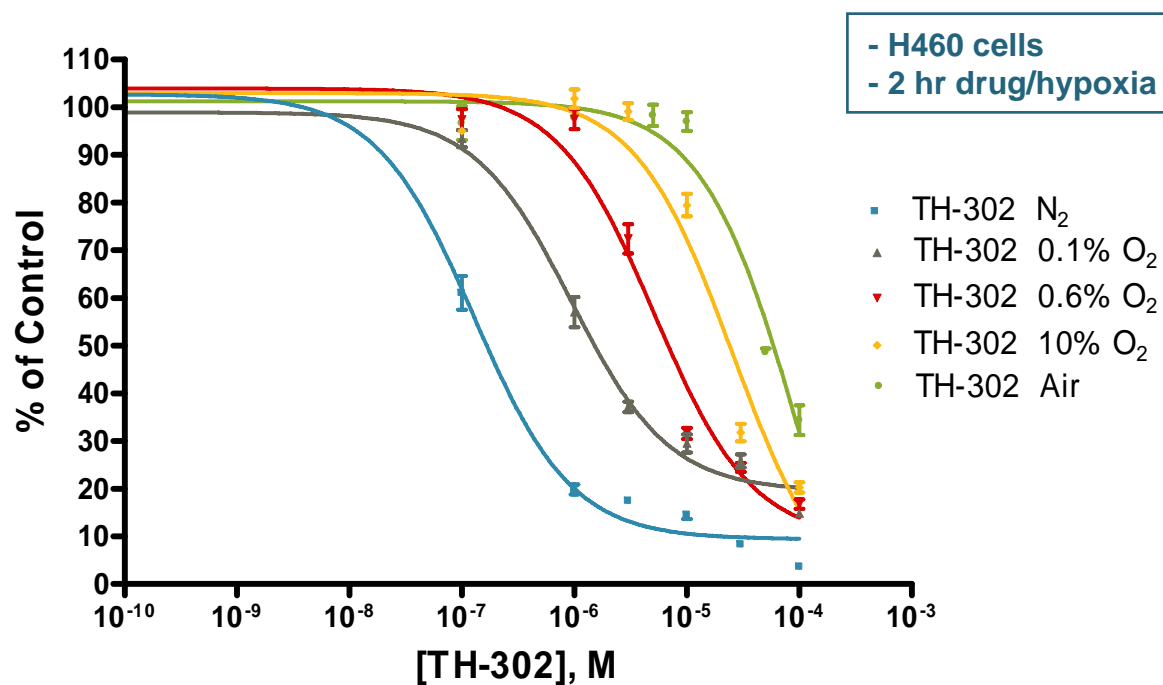
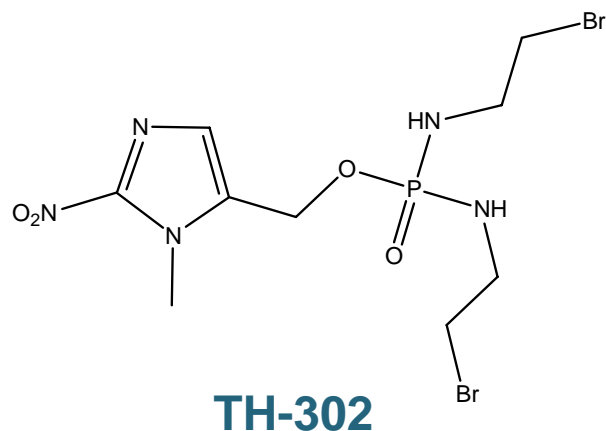
Grow for  
7-10 days



Count Colonies

# TH-302 Hypoxia-Selective Cytotoxicity

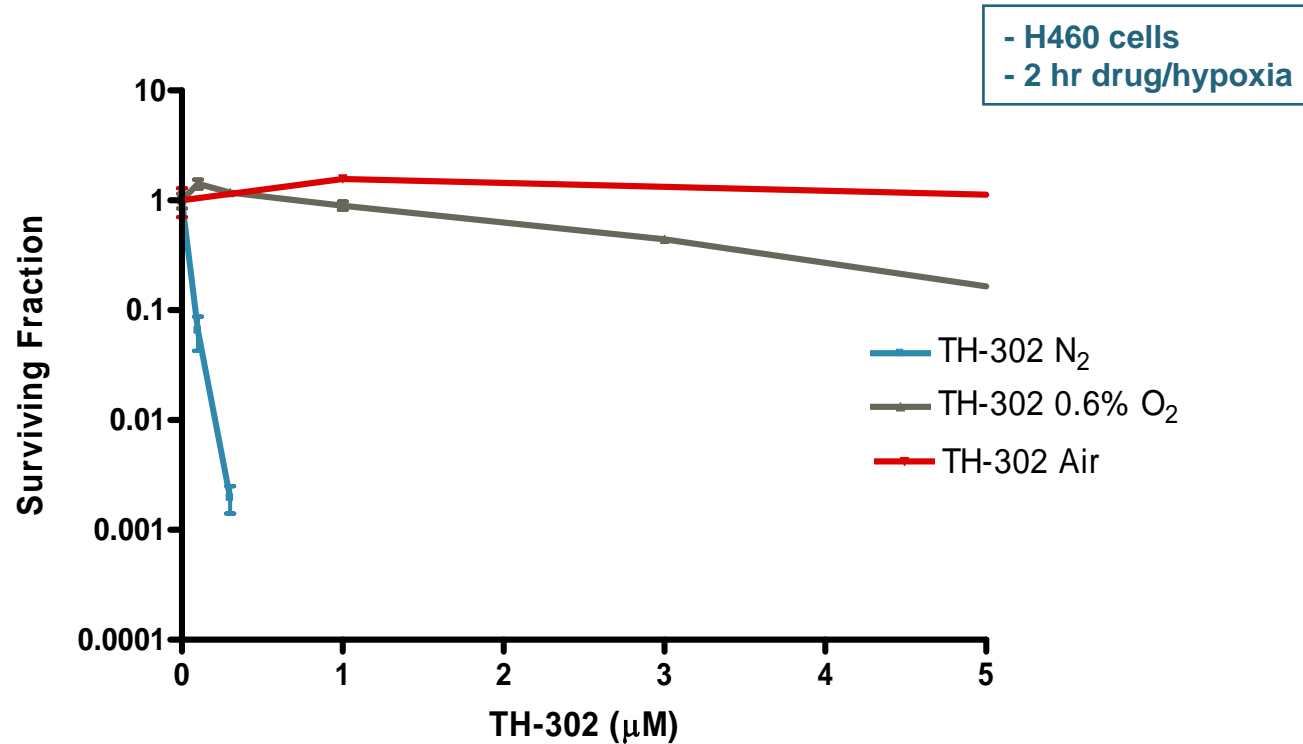
~500x selectivity between anoxia and normoxia



	N <sub>2</sub>	0.1% O <sub>2</sub>	0.6% O <sub>2</sub>	10% O <sub>2</sub>	Air
IC <sub>50</sub> (μM)	<b>0.1</b>	<b>1</b>	<b>5</b>	<b>25</b>	<b>55</b>

# TH-302 Hypoxia-Selective Cytotoxicity

Demonstrated with a clonogenic survival assay



	N <sub>2</sub>	0.6% O <sub>2</sub>	Air
IC <sub>90</sub> (μM)	<b>0.01</b>	<b>5</b>	<b>30</b>

# TH-302 *in vitro* efficacy

Cell lines exhibiting sensitivity and hypoxia selectivity

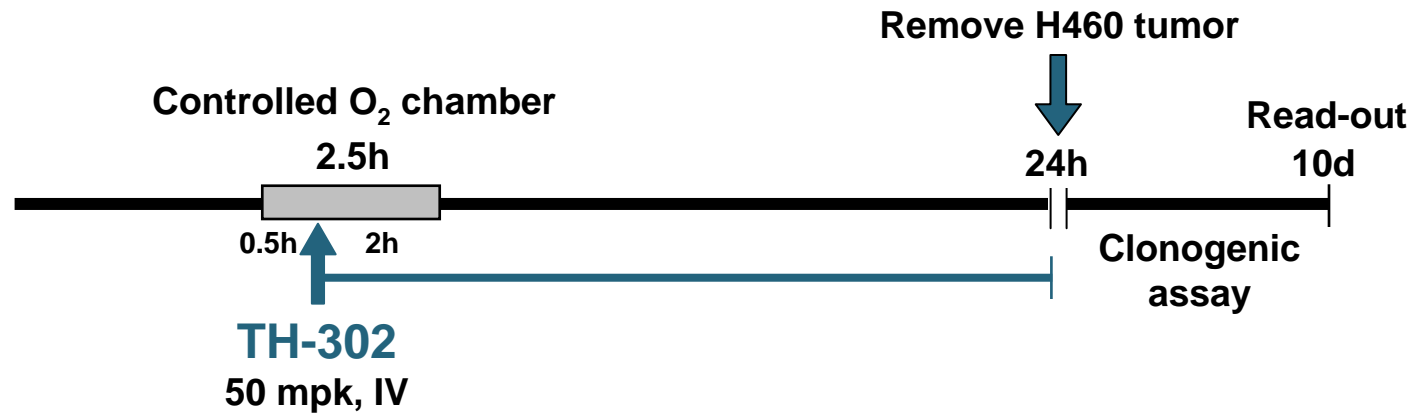
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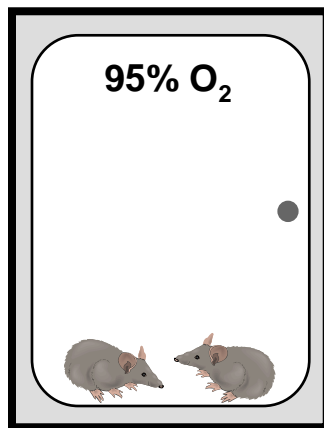
- ▶ **H460** human lung cancer cells (**NSCLC**)
  - ▶ **HT29** human **colon** cancer cells
  - ▶ **PC3** human **prostate** cancer cells
  - ▶ **MIA PaCa-2** human **pancreatic** cancer cells
  - ▶ **MES-SA** human **uterine sarcoma** cells
  - ▶ **ACHN** human **renal** cancer cells
  - ▶ **H69** human small cell lung cancer cells (**SCLC**)
  - ▶ **HEK 293** human embryonal kidney cells
  - ▶ **HCT116** human **colon** cancer cells
-

# TH-302

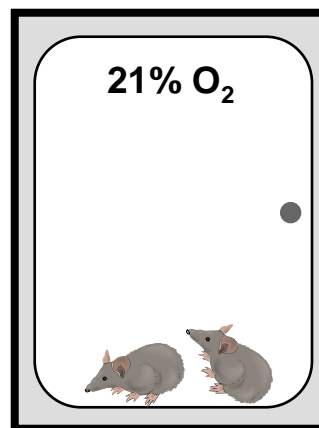
## Oxygen concentration dependence of cytotoxicity *in vivo*



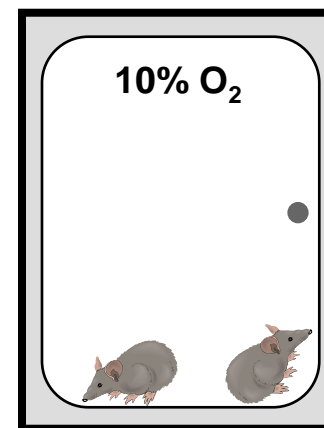
### Hyperoxia



### Normoxia

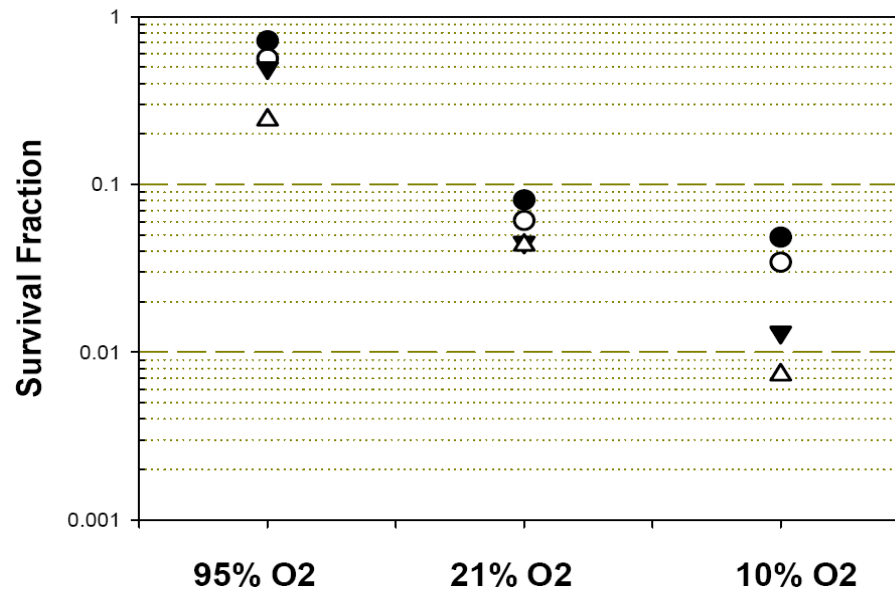


### Hypoxia



# TH-302

Demonstration of hypoxia-enhanced cytotoxicity *in vivo*



# TH-302 *in vivo* efficacy

## Summary of preclinical experiments

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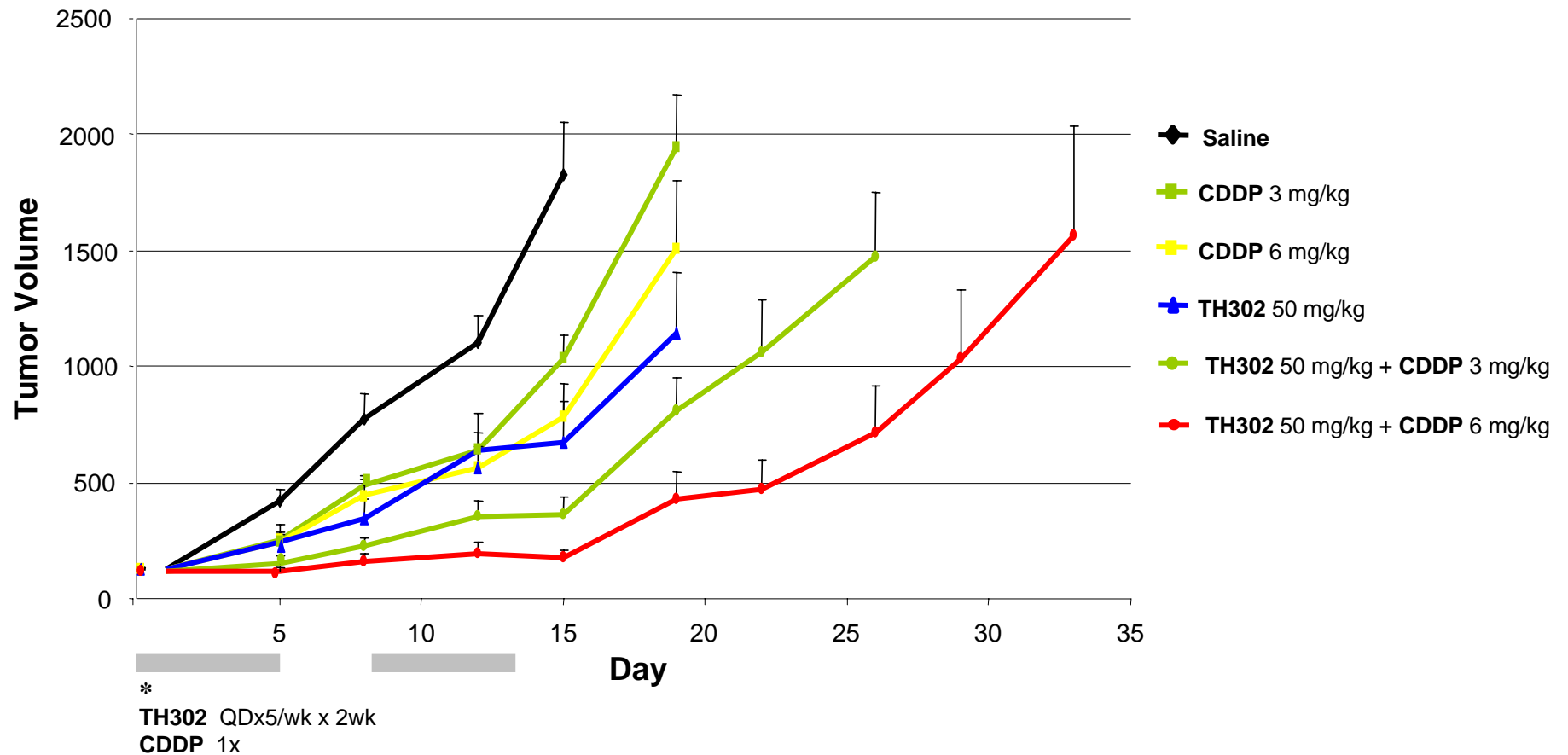
- ▶ Initial screening model
    - Ectopic lung H460 xenograft +/- Cisplatin
  
  - ▶ Follow-up orthotopic xenografts
    - Pancreatic MIA PaCA-2 +/- Gemcitabine
    - Prostate PC-3 +/- Taxotere
  
  - ▶ Regimen, schedule, route optimization
    - Lung H460 and colon HT29 ectopics
  
  - ▶ Other tumor types and exploring combinations with different conventional chemotherapeutic drugs
-

# TH-302 *in vivo* – H460 Ectopic Xenograft

Initial demonstration of preclinical efficacy



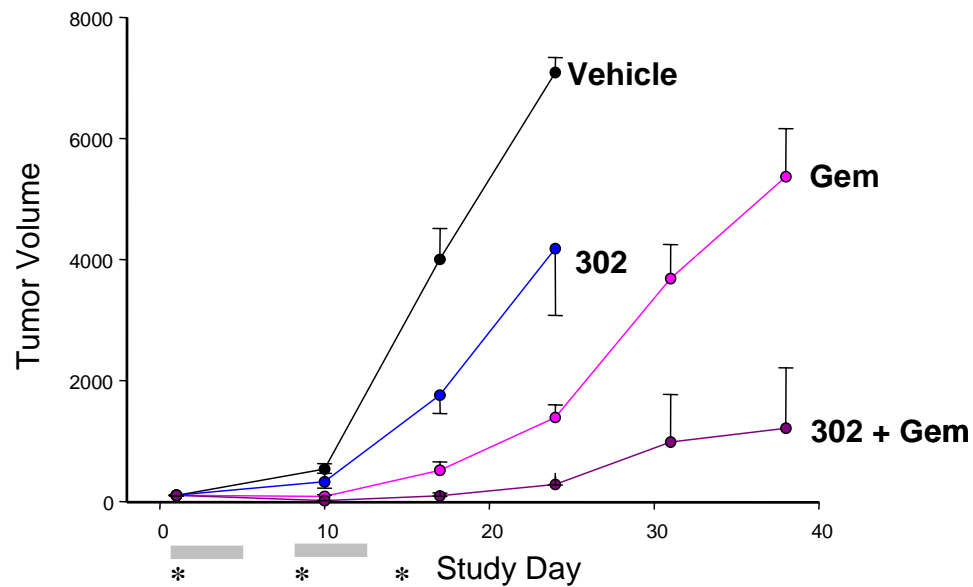
## Disease Progression





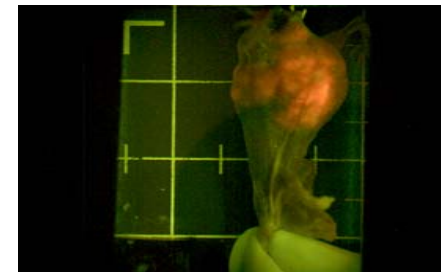
# TH-302 *in vivo* - MIA PaCa-2 Orthotopic

## Preclinical pancreatic cancer efficacy

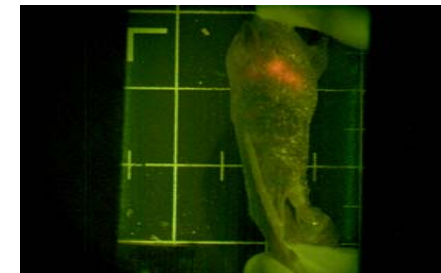


TH-302 QDx5/wk x 2wk

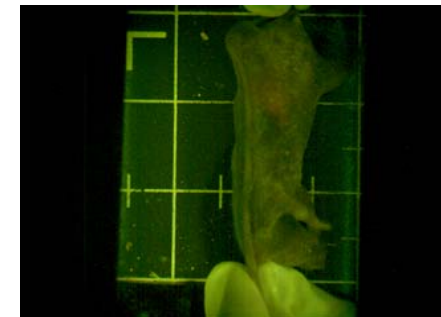
Gem Q7D x 3wk



Vehicle control



Gem



Gem + TH-302

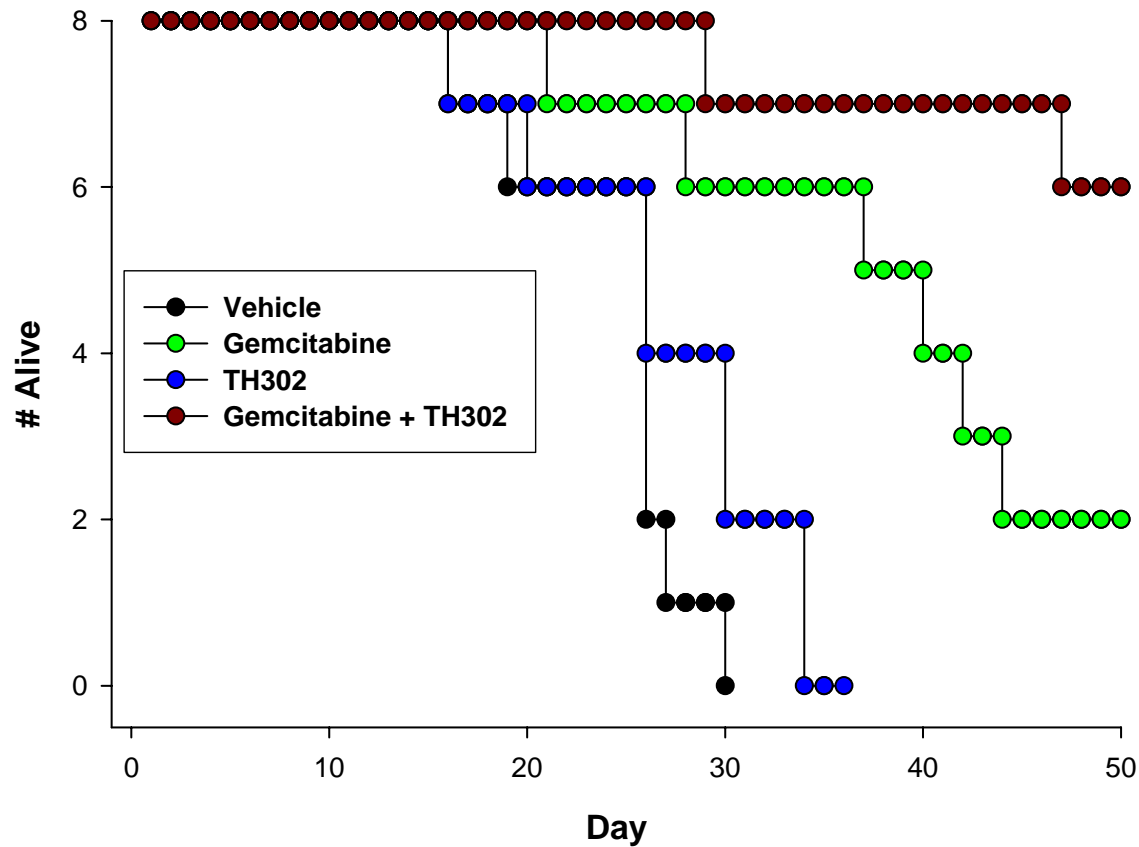
RFP (red fluorescent protein) expressing MIA PaCa-2 cells

# TH-302 *in vivo* - MIA PaCa-2 Orthotopic

Preclinical pancreatic cancer efficacy



## Survival (Kaplan-Meier plot)

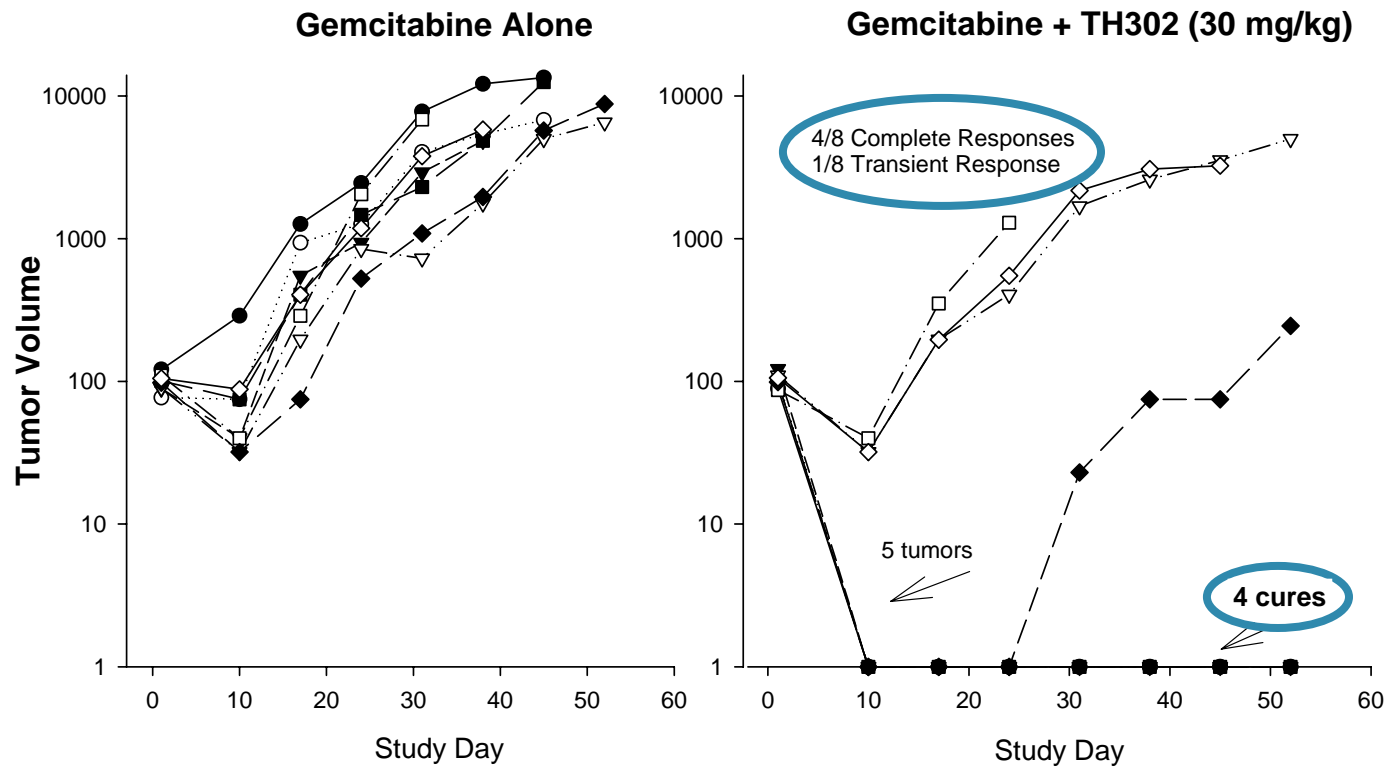


# TH-302 *in vivo* - MIA PaCa-2 Orthotopic

Preclinical pancreatic cancer efficacy

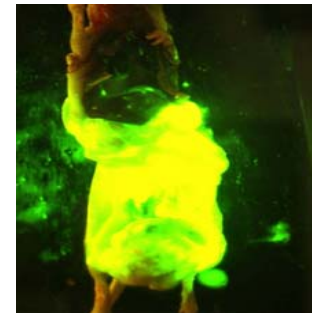
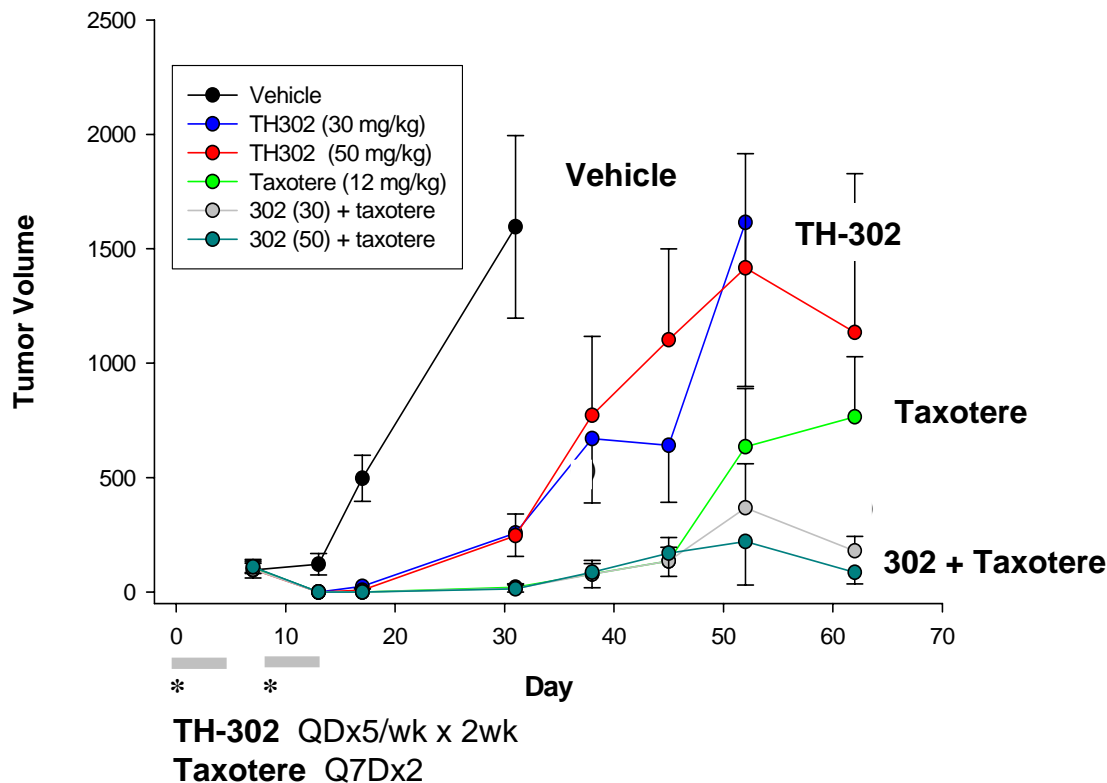


## Objective responses

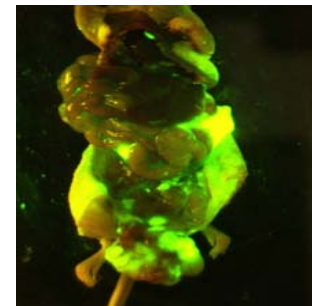


# TH-302 *in vivo* efficacy

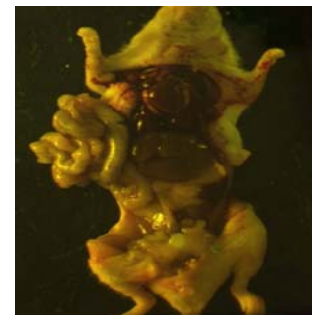
Activity in a second orthotopic model (PC3 prostate model)



Vehicle control



Taxotere



Taxotere + TH-302

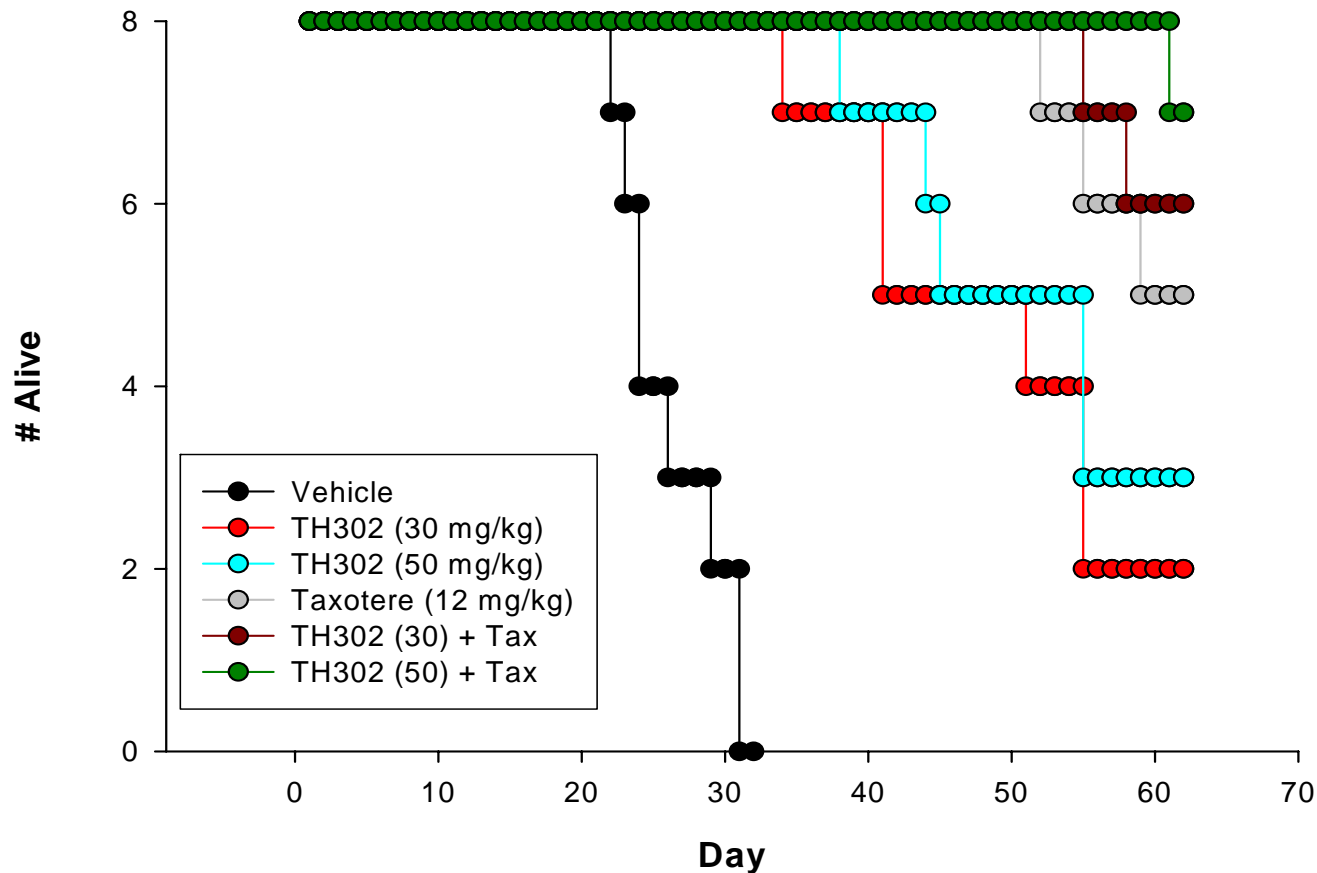
GFP (green fluorescent protein) expressing PC3 cells

# HAP TH-302 *in vivo* efficacy

Activity in a second orthotopic model (PC3 prostate model)



## Survival (Kaplan-Meier plot)

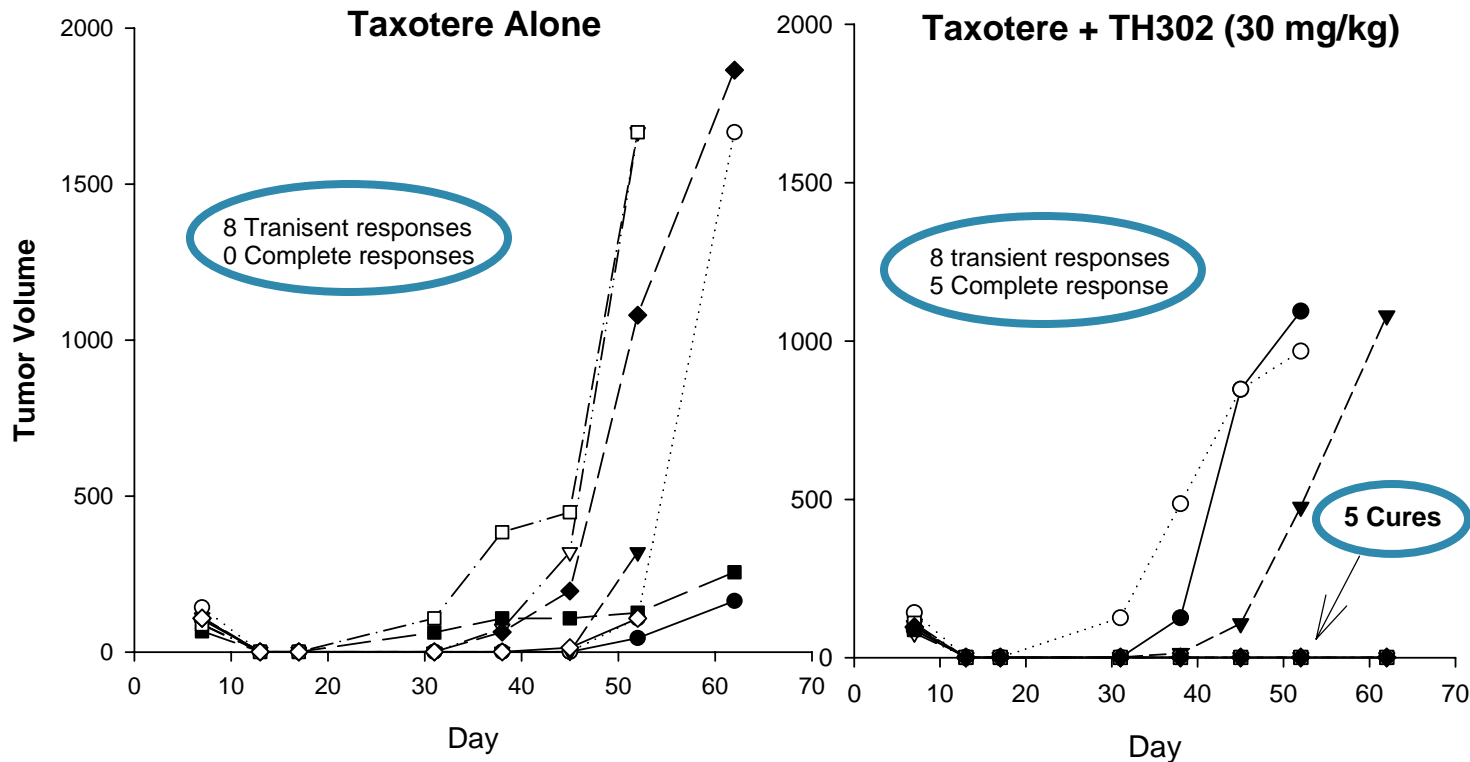


# HAP TH-302 *in vivo* efficacy

Activity in a second orthotopic model (PC3 prostate model)



## Objective responses



# Additional Preclinical Studies

For helping guide clinical usage

---



- ▶ Route of administration
    - IV vs. PO
  
  - ▶ Dosing regimen
    - QDx5 vs. Q3Dx5 vs. Q7Dx3 vs. constant infusion 7Dx2
  
  - ▶ Co-administration and sequencing
    - Gemcitabine, Cisplatin, Carboplatin, 5FU,
    - Irinotecan (CPT-11), Taxol, Taxotere, Radiation
  
  - ▶ Tumor type
    - Colon (*HT29, HCT116*)
    - Ovarian (*IGROV-1, SKOV-3*)
    - Cervical (*SiHa*)
    - Lung (*H460*)
    - Pancreatic (*MIA PaCa-2*)
    - Prostate (*PC-3*)
-

# Additional Preclinical Studies

For helping guide clinical usage



## ► Route of administration

- IV vs. PO

**IV best**

## Dosing regimen

- QDx5 vs. Q3Dx5 vs. Q7Dx3 vs. constant infusion 7Dx2

## Co-administration and sequencing

- Gemcitabine, Cisplatin, Carboplatin, 5FU,
- Irinotecan (CPT-11), Taxol, Taxotere, Radiation

## Tumor type

- Colon (*HT29, HCT116*)
- Ovarian (*IGROV-1, SKOV-3*)
- Cervical (*SiHa*)
- Lung (*H460*)
- Pancreatic (*MIA PaCa-2*)
- Prostate (*PC-3*)



# Additional Preclinical Studies

For helping guide clinical usage



Route of administration

- IV vs. PO

► Dosing regimen *(at equal total dose)*

- QDx5 vs. Q3Dx5 vs. Q7Dx3 vs. constant infusion 7Dx2

**Efficacy similar, but weekly high dose results best**

Co-administration and sequencing

- Gemcitabine, Cisplatin, Carboplatin, 5FU,
- Irinotecan (CPT-11), Taxol, Taxotere, Radiation

Tumor type

- Colon (*HT29, HCT116*)
- Ovarian (*IGROV-1, SKOV-3*)
- Cervical (*SiHa*)
- Lung (*H460*)
- Pancreatic (*MIA PaCa-2*)
- Prostate (*PC-3*)

# Additional Preclinical Studies

For helping guide clinical usage



Route of administration

- IV vs. PO

Dosing regimen

- QDx5 vs. Q3Dx5 vs. Q7Dx3 vs. constant infusion 7Dx2

► Tumor type and drug combination

- HT-29 (colon) +/- 5FU
- HT-29 (colon) +/- irinotecan (CPT-11)
- HT-29 (colon) +/- cisplatin
- IGROV-1 (ovarian) +/- cisplatin
- SKOV-3 (ovarian) +/- taxol
- SiHa (cervical) monotherapy only
- H460 (lung) +/- cisplatin
- H460 (lung) +/- carboplatin
- H460 (lung) +/- Taxol
- H460 (lung) +/- radiation

**TH-302 efficacy observed in all models and combinations examined to date**

# Preclinical Development

TH-302 then underwent a variety of IND-enabling studies

---



- ▶ PK in monkeys, rats, dogs, mice
  - ▶ Microsomal stability
  - ▶ Mass balance in rats / dogs
  - ▶ Metabolite profiling
  - ▶ QWBA (quantitative whole body autoradiography)
  - ▶ Plasma protein binding
  - ▶ Drug resistance profiling
  - ▶ Acute tox
  - ▶ Sub-chronic tox
-

# Preclinical Development

TH-302 then underwent a variety of IND-enabling studies

---



PK in monkeys, rats, dogs, mice

▶ **Microsomal stability**

Mass balance in rats / dogs

Metabolite profiling

▶ **QWBA (quantitative whole body autoradiography)**

Plasma protein binding

▶ **Drug resistance profiling**

Acute tox

Sub-chronic tox

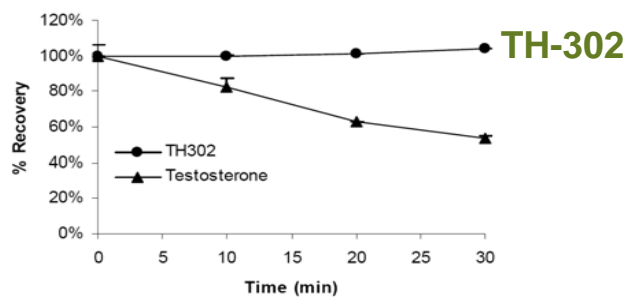
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# TH-302 Metabolic Stability

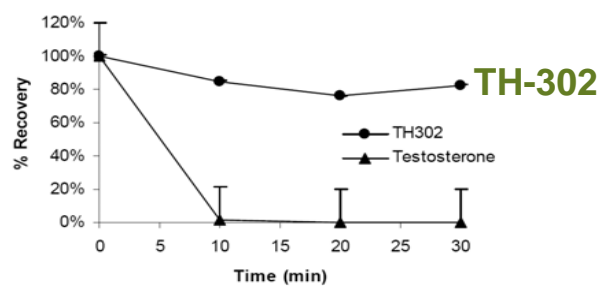
Stable in mouse, rat, dog, monkey and human liver microsomes



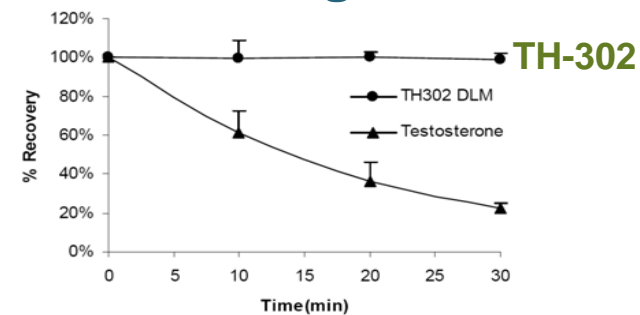
## Mouse



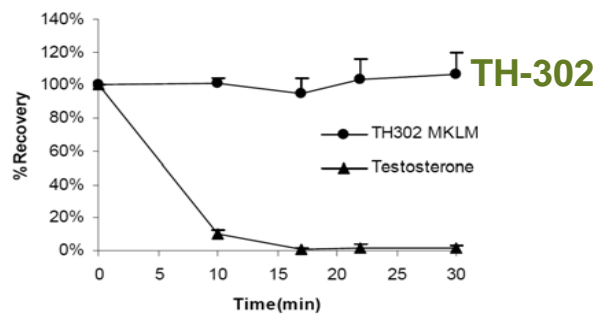
## Rat



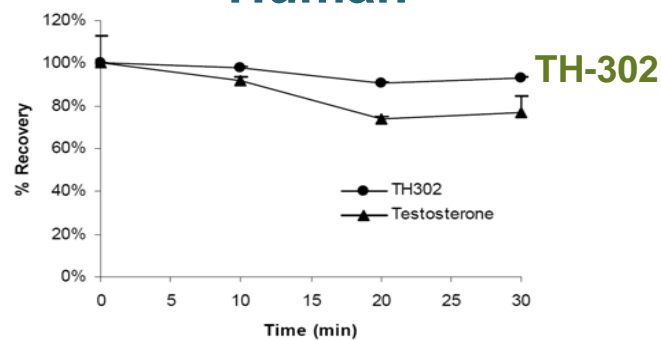
## Dog



## Monkey

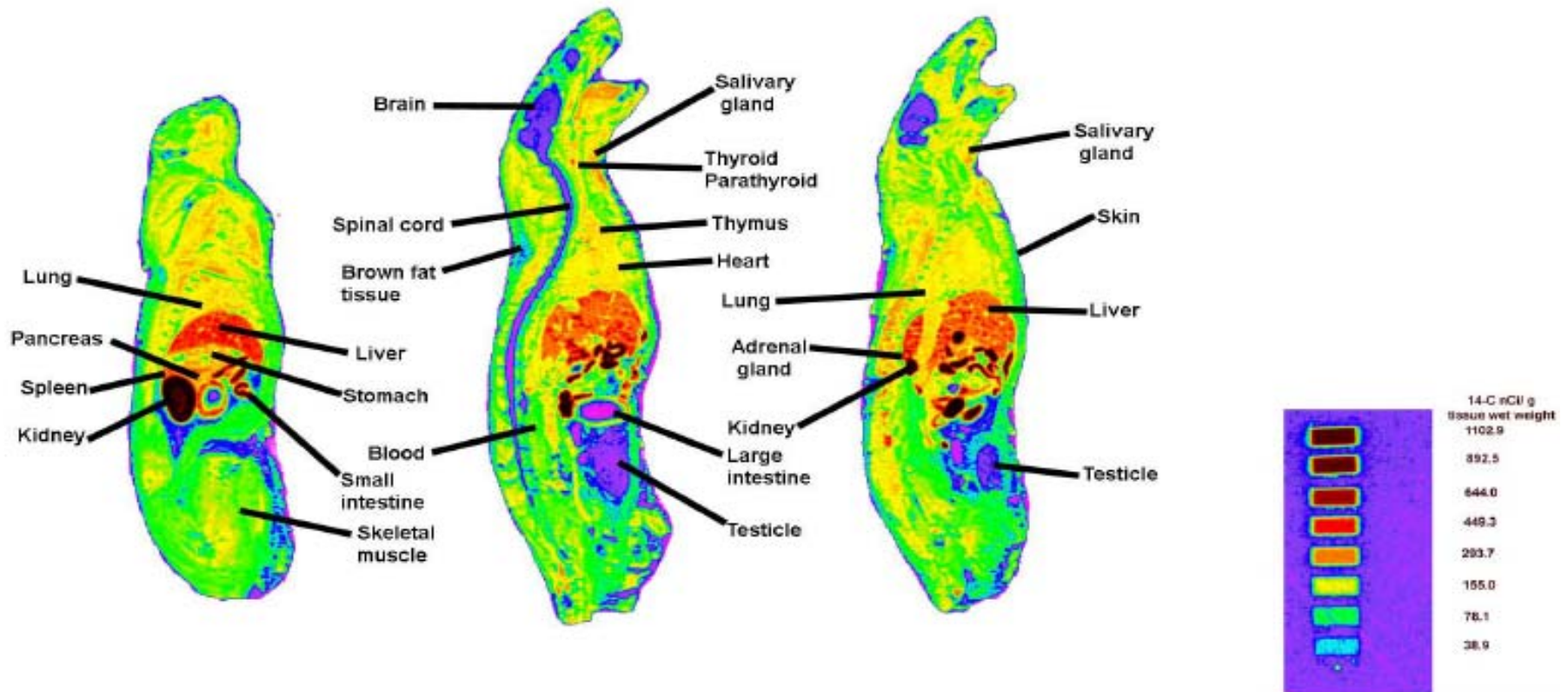


## Human



# TH-302 Biodistribution

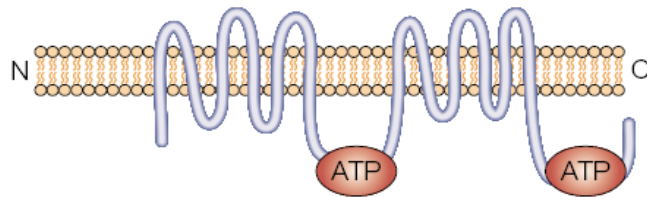
[<sup>14</sup>C]-TH-302 distributes rapidly, and broadly, in the rat



10 min post 50 mpk IV dose

# TH-302 Drug Resistance Profiling

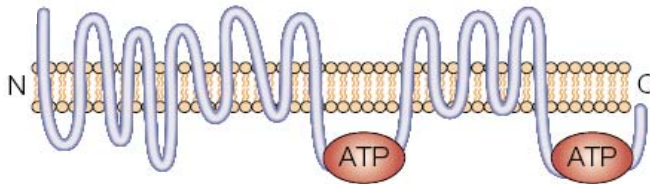
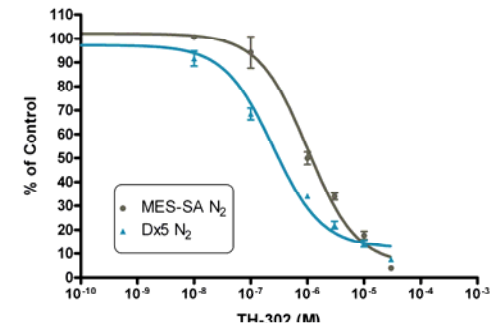
Not a Substrate for Clinically Relevant Drug Resistance Pumps



## MDR1

MDR1 (ABCB1)  
MRP4 (ABCC4)  
MRP5 (ABCC5)  
MRP7 (ABCC1)  
BSEP/SPGP (ABCB11)

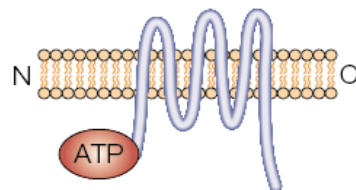
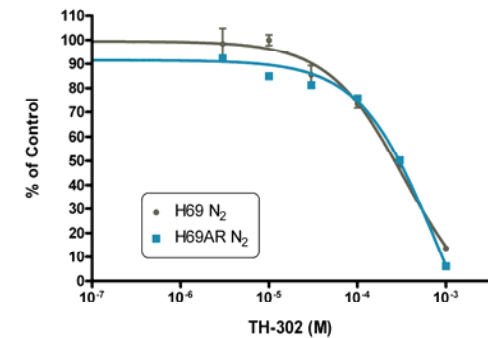
Taxol >500x



## MRP1

MRP1 (ABCC1)  
MRP2 (ABCC2)  
MRP3 (ABCC3)  
MRP6 (ABCC6)

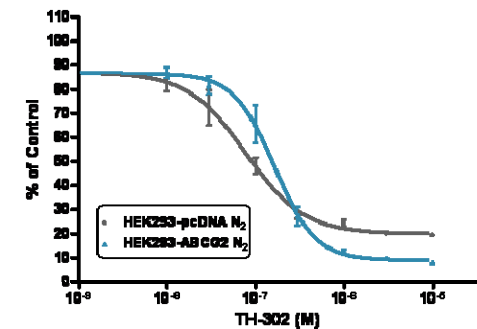
Daunorubicin >10x



## BCRP

MXR/BCRP/ABC-P  
(ABCG2)

Mitoxantrone >30x



# TH-302 Phase 1 Clinical Trial

## Preliminary design

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- ▶ Phase 1, multi-center, open-label, dose-escalation in patients with solid tumors
  - ▶ Primary objectives are to establish recommended dose and to determine dose-limiting toxicities
  - ▶ Secondary endpoints are pharmacokinetics and tumor response
  - ▶ ~50 patients will be enrolled
  - ▶ Plan to start study Q3 2007
-



# Acknowledgements

## TH-302 Discovery and Preclinical Development

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- ▶ Chemistry
    - M. Matteucci
    - J.-X. Duan
    - H. Jiao
    - J. Kaizerman
    - T. Stanton
  - ▶ Biology
    - J. Evans
    - L. Lan
    - F. Meng
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