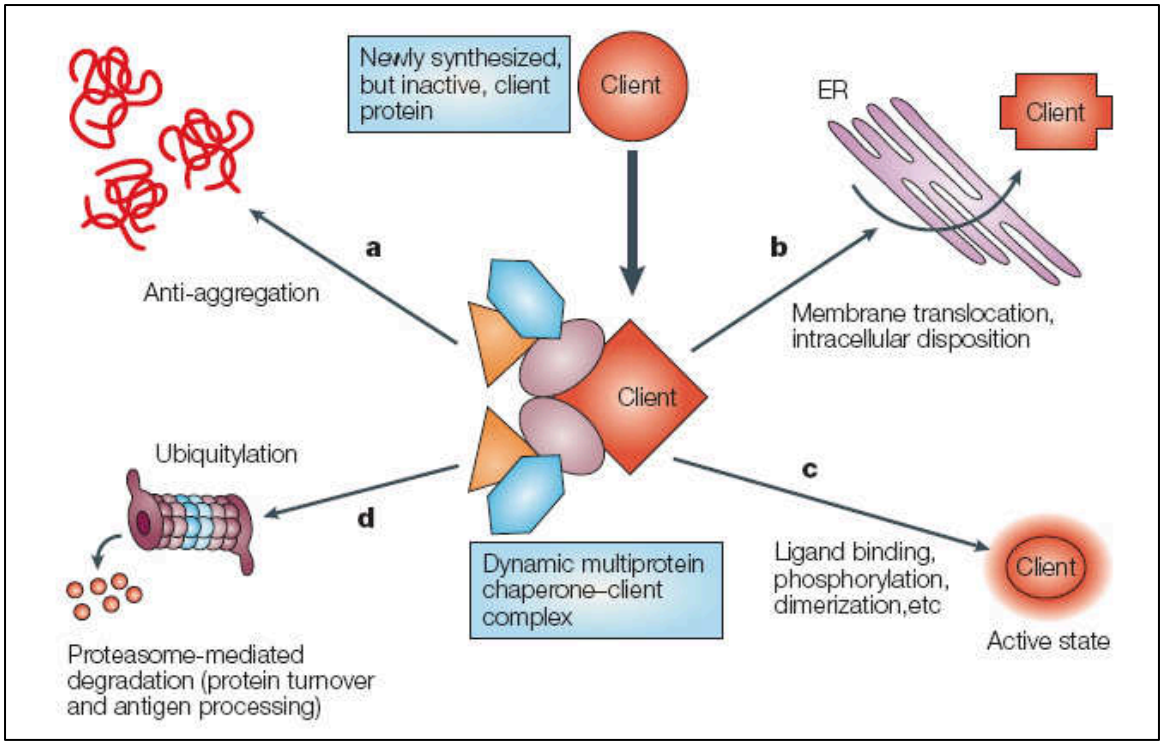
The novel Hsp90 inhibitor STA-1474 exhibits biologic activity against osteosarcoma

February 15, 2009

Jennifer K. McCleese, B.S., D.V.M
Graduate Research Associate/Clinical Pathology Resident
Department of Veterinary Biosciences
The Ohio State University

Heat shock proteins

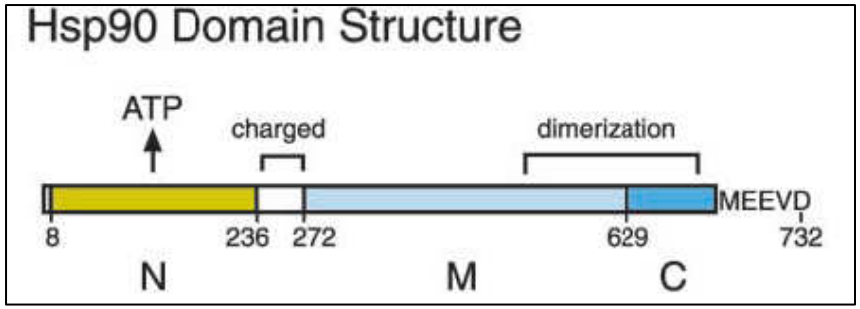
- Required for cell survival during stress
- Named according to the relative molecular mass of their encoded proteins.
- Function as molecular chaperones

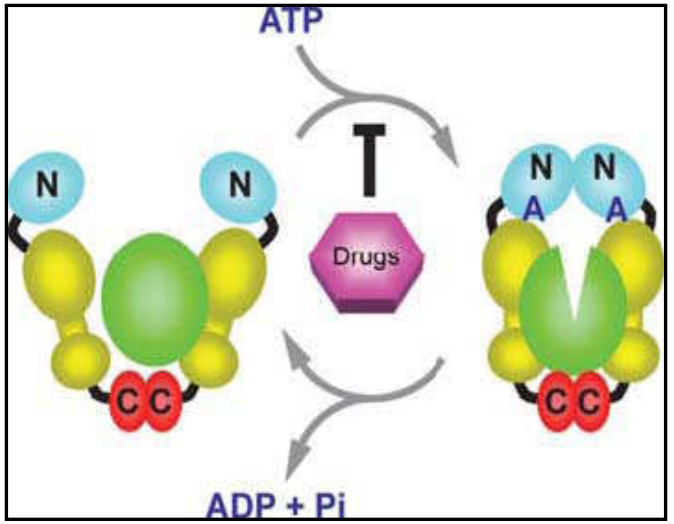


L. Whitesell, and Lindquist, S. Hsp90 and the Chaperoning of Cancer. Nature Reviews 5: Oct 2005

Hsp90 function

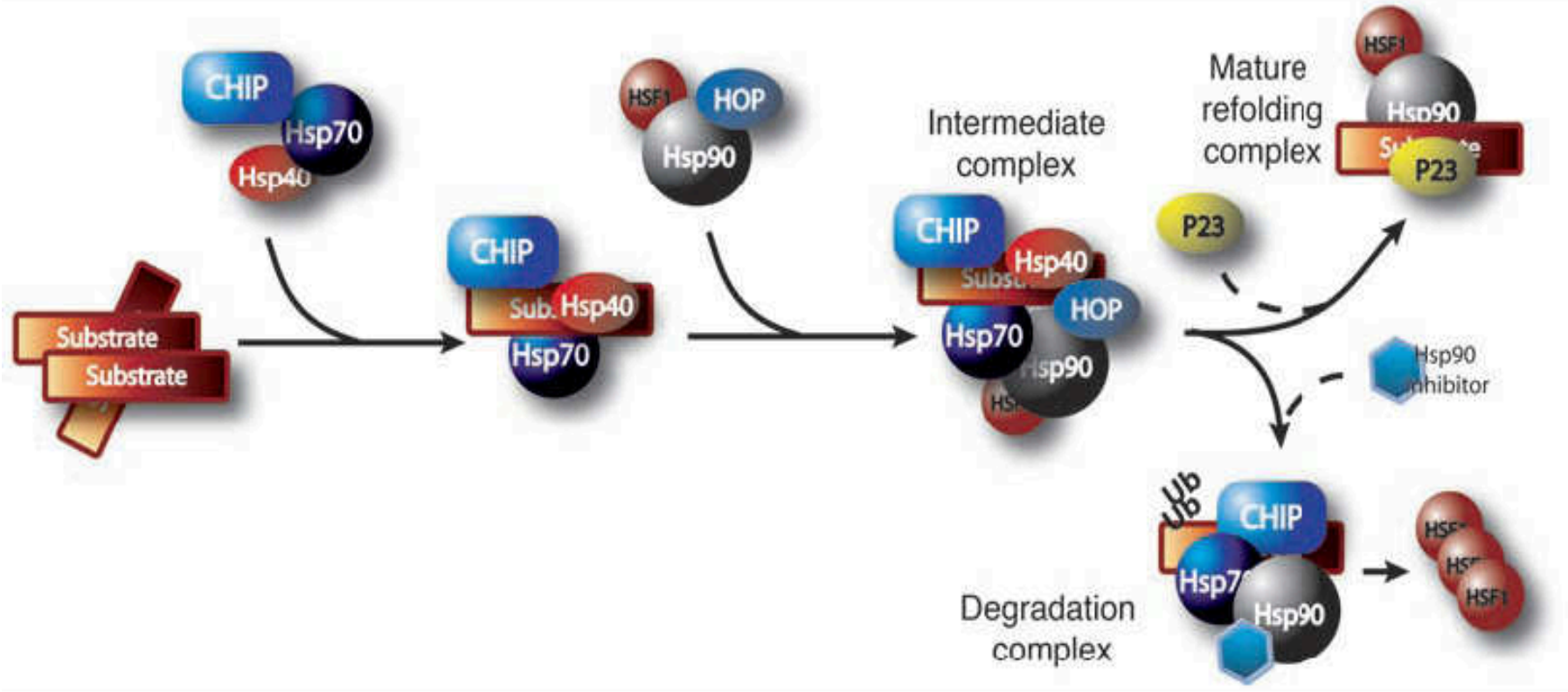
- Molecular chaperone which promotes the correct folding, maturation, and stabilization of client proteins
- Clients
  - Kinases
  - Hormone receptors
  - Transcription factors
- ATP binding and hydrolysis are required for the refolding and release of the native protein from the chaperone complex.





Young, JC, Moarefi, I, and Hartl, F. J of Cell Biology 154, 2001

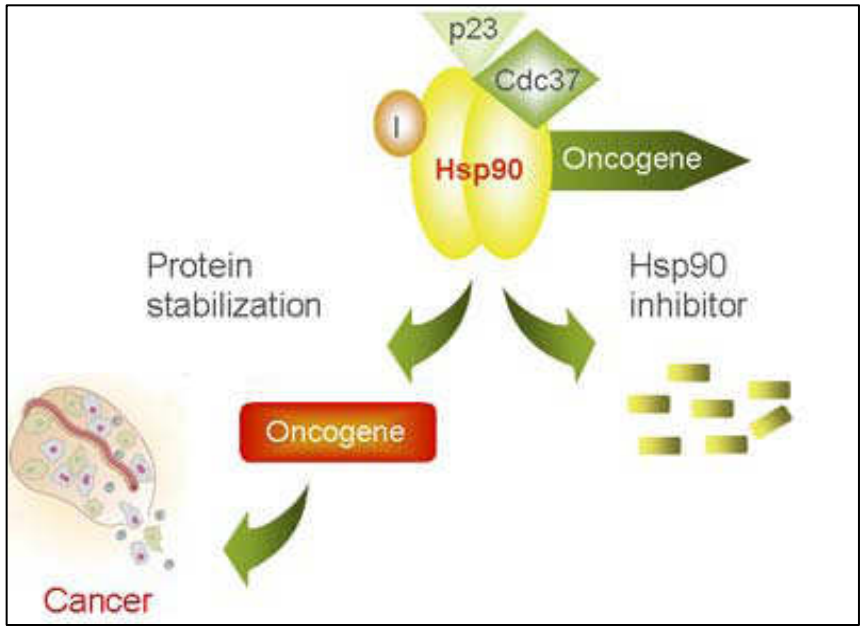
Hsp90 active multi-chaperone complex



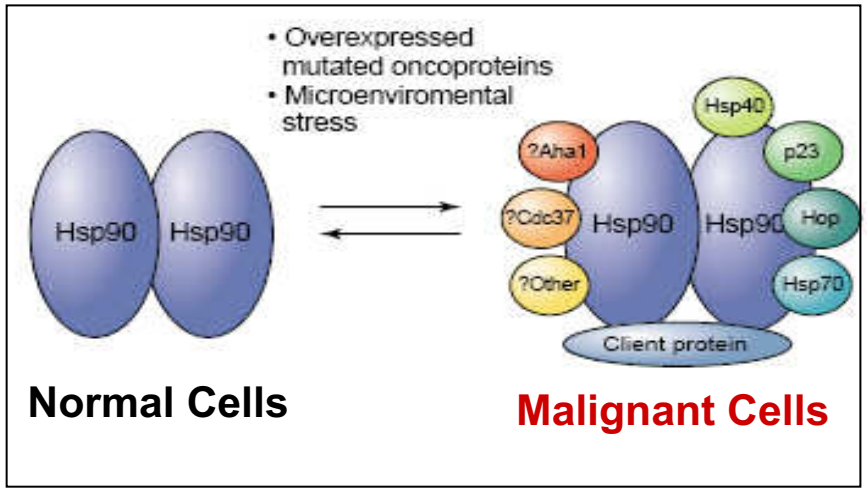
Dickey, Chad A. The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. Journal of Clinical Investigation. 2007

Hsp90 as a target in cancer therapy

- Clients
  - Many are known oncogenes: EGFR, Bcr-Abl, Akt, Kit, Met.
  - "Buffers" over-expressed or mutant proteins



- Selectivity for malignant vs. normal cells
  - Super-chaperone complex
  - Higher affinity for Hsp90 inhibitor and ATPase activity

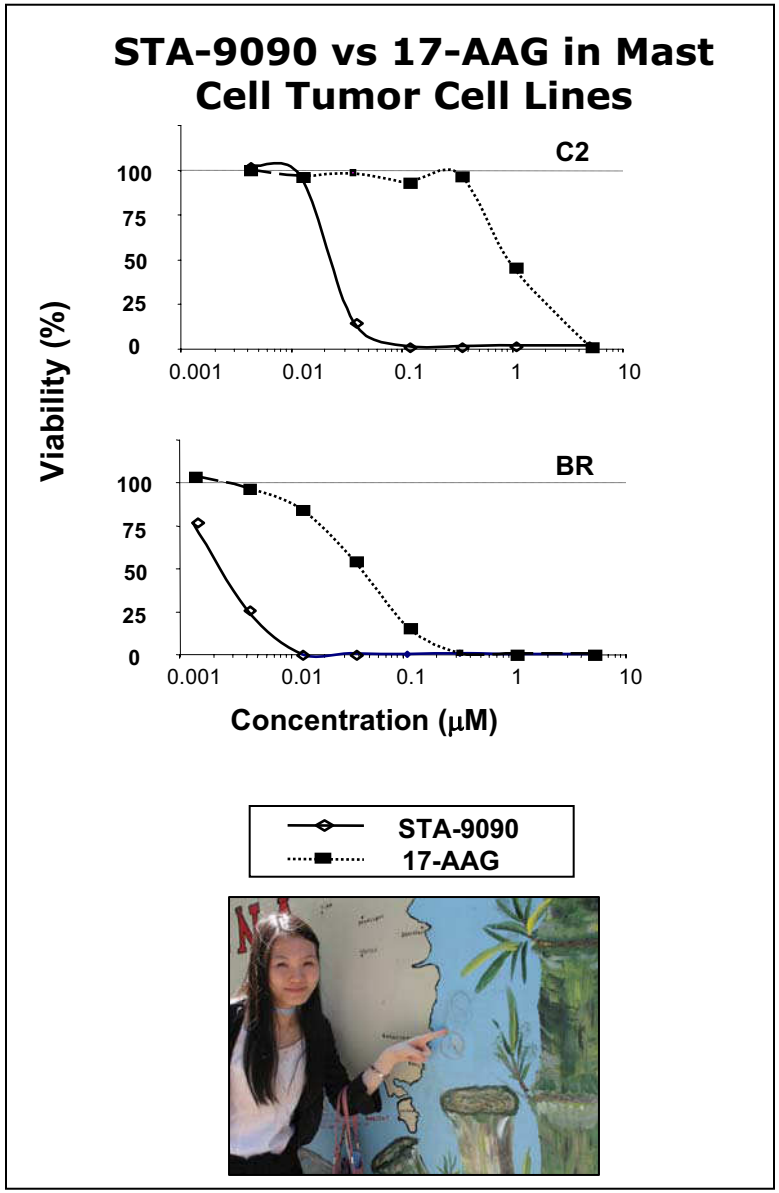


Hsp90 inhibitors

- Hsp90 inhibitors previously tested in clinical trials
  - Geldanamycin
  - 17-AAG
  - 17-DMAG
- Limitations
  - Low solubility
  - Liver toxicity
  - Substrate for p-glycoprotein export pump

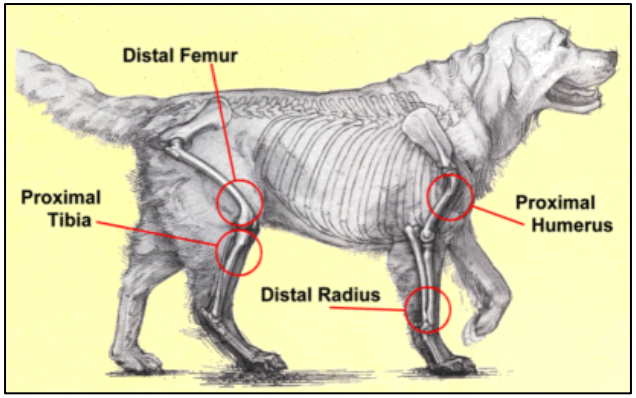
STA-1474 (Synta)

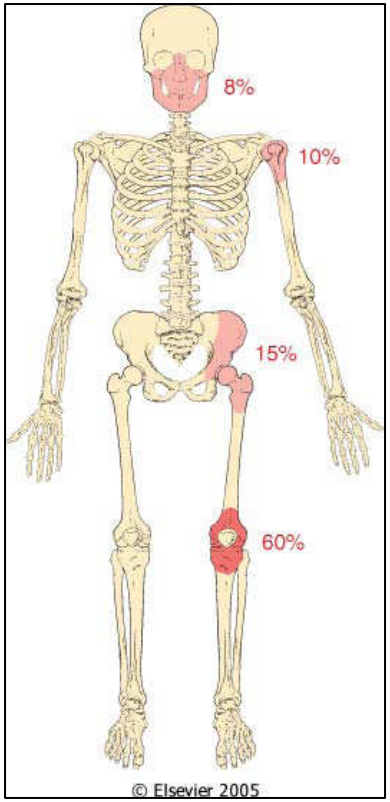
- Novel triazolone compound
- Potent inhibitor of Hsp90 that binds in the ATP-binding domain of the N-terminus of Hsp90
- Metabolized *in vivo* to STA-9090 which has 10-100-fold greater potency compared to 17-AAG and 17-DMAG



Osteosarcoma

- **Incidence**
  - Most common primary bone tumor in dogs and children
    - 10,000 vs 1,000 new cases/year
- **Clinical Presentation**
  - Osteolytic/proliferative lesion of metaphases of long bones
  - Micrometastases present at diagnosis
  - Metastatic OSA extremely resistant to chemotherapy
- **Prognosis**
  - <20% 2 year survival rate for dogs
  - 30% children die despite aggressive treatment



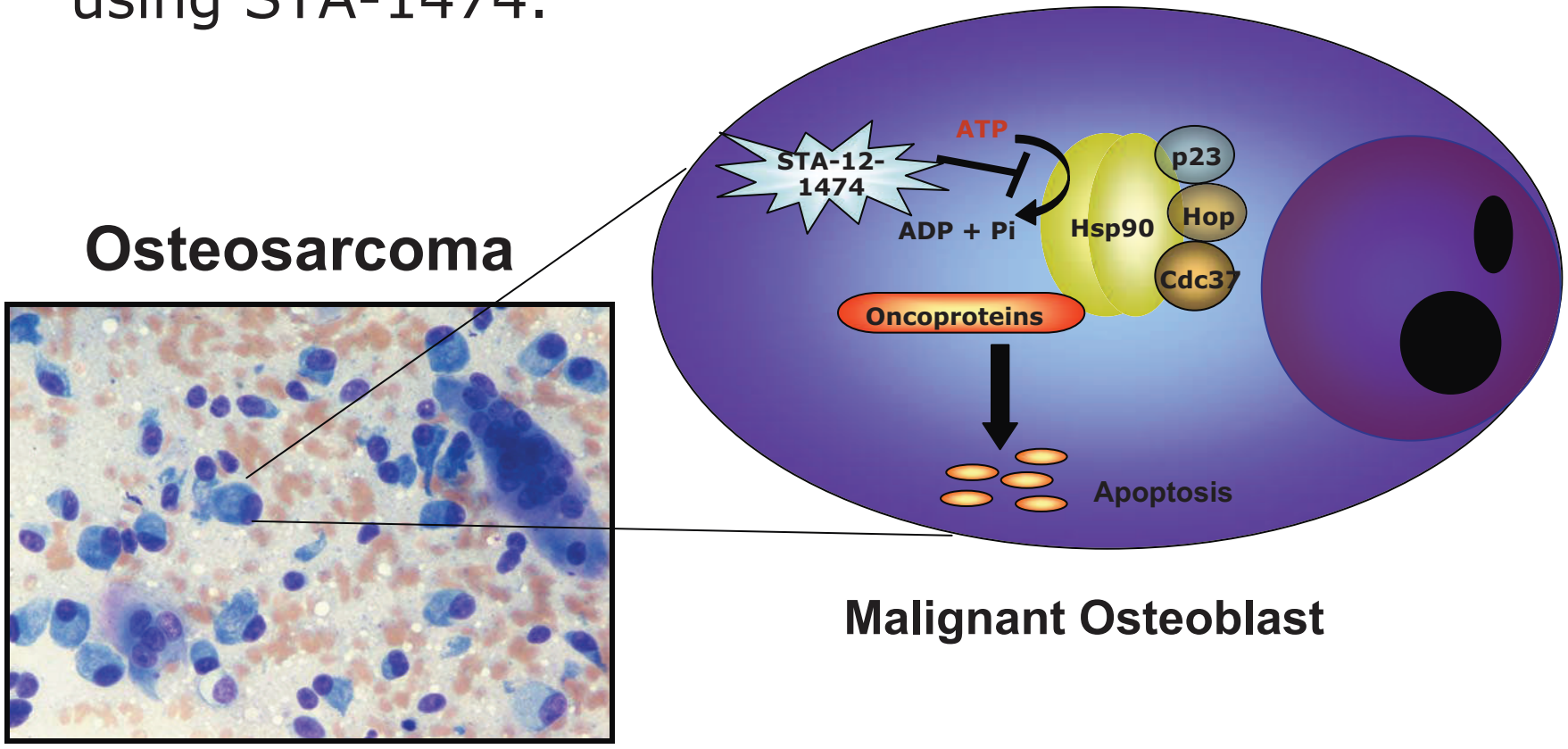


Objective

Evaluate the biologic activity of a novel Hsp90 inhibitor, STA-1474 (Synta Pharmaceuticals) in the treatment of osteosarcoma.

Hypothesis

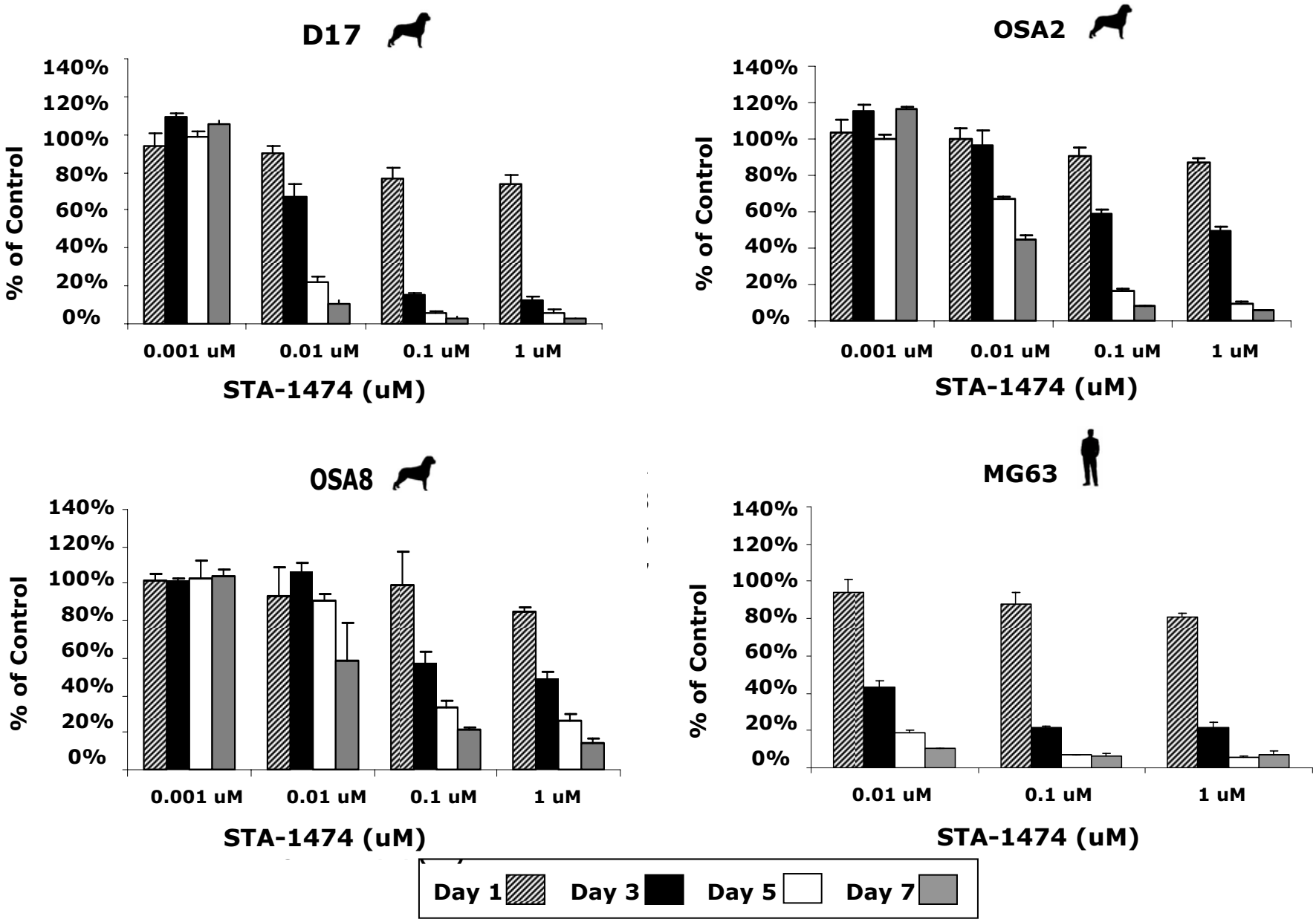
Hsp90 exists in a multi-chaperone active complex in OSA cells, allowing selective targeting of malignant cells, promoting client protein down-regulation and cell death upon Hsp90 inhibition using STA-1474.



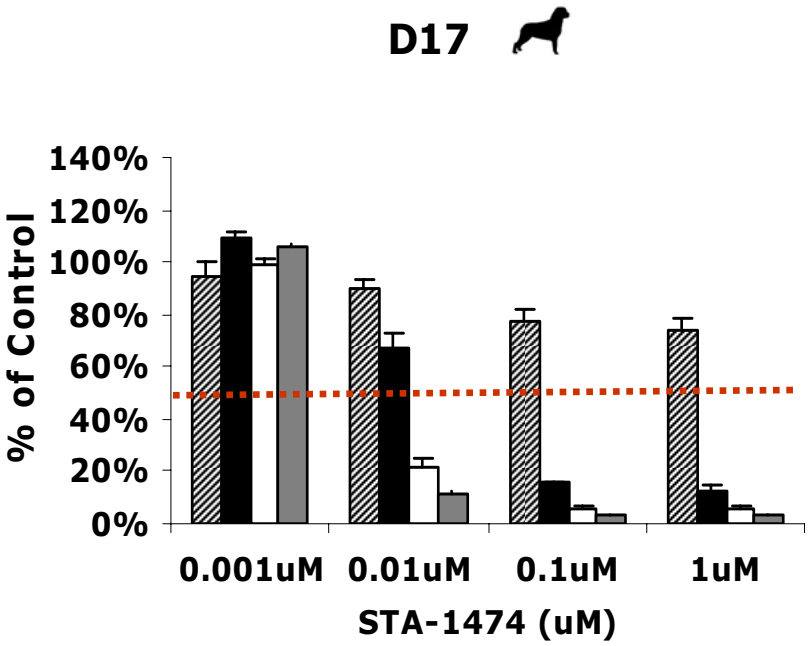
Specific aims

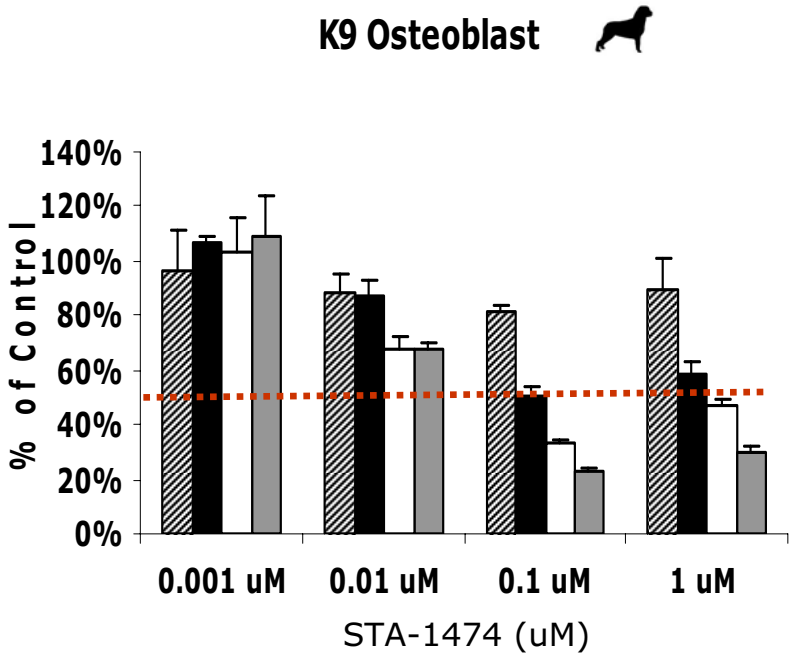
- **Evaluate the effects of STA-1474 on cell viability, cell survival, and signal transduction in canine OSA cell lines.**
- **Assess selectivity of Hsp90 inhibition for malignant OSA cells versus normal canine osteoblasts.**
- **Evaluate the potential anti-tumor effects of STA-1474 *in vivo* using a mouse xenograft model.**

STA-12-1474 inhibits OSA viability



K9 OSA is more sensitive to Hsp90 inhibition

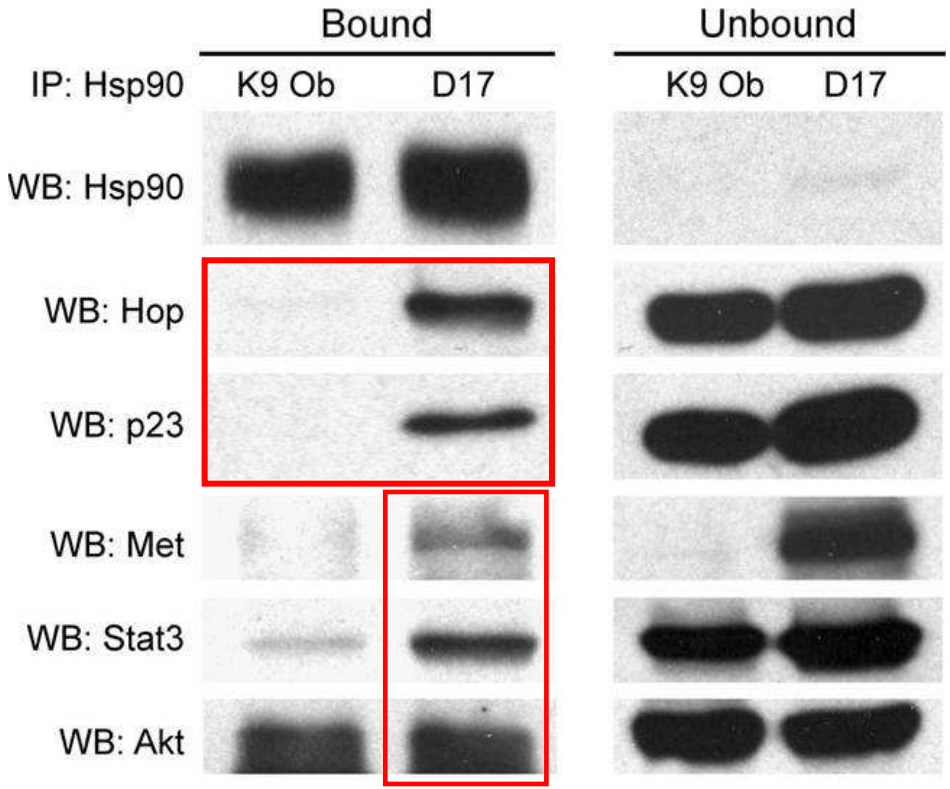




Osteoblasts have ~2-10 fold higher IC50 at any given time compared to OSA cells

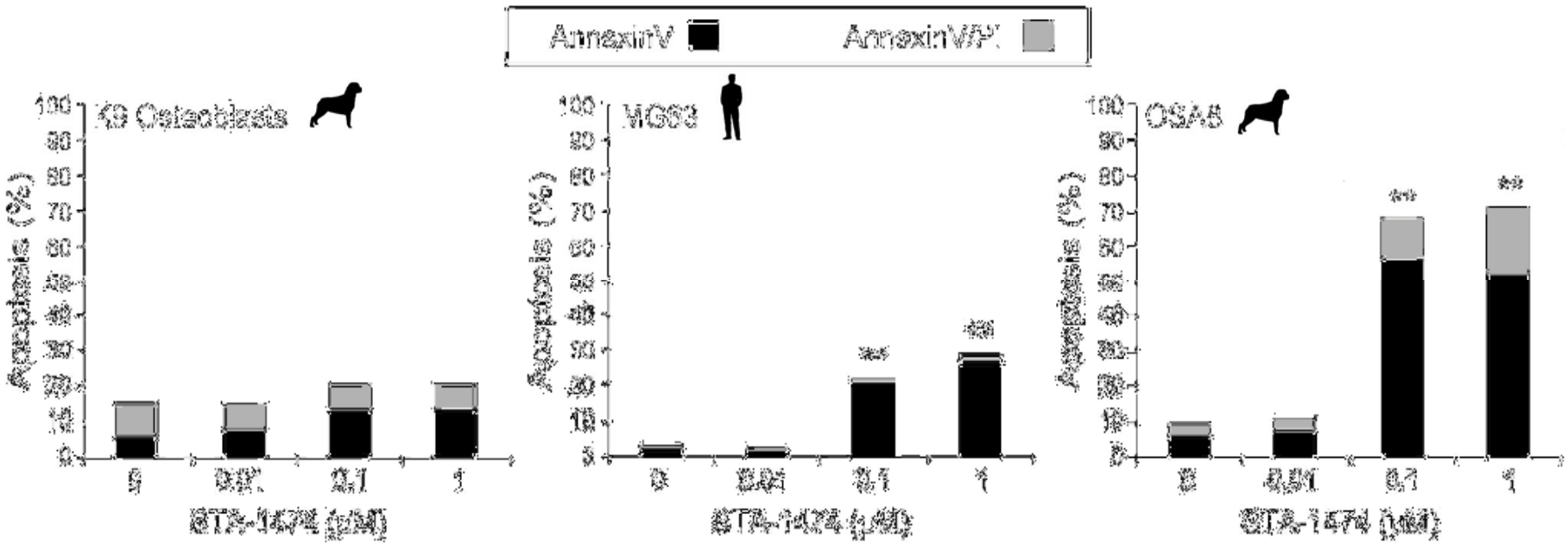
	IC50 K9/IC50 OSA (fold)
Day 3 % of viability	24.88X
Day 5 % of viability	12.66X
Day 7 % of viability	3.67X

Hsp90 exists in a super-chaperone complex in OSA



- **Hsp90 is associated with co-chaperones p23 and Hop, indicative of the active super-chaperone complexed Hsp90 in K9 OSA vs normal K9 osteoblasts.**
- **Hsp90 is associated with Akt, Stat3, and Met in K9 OSA cells.**

STA-1474 induces apoptosis

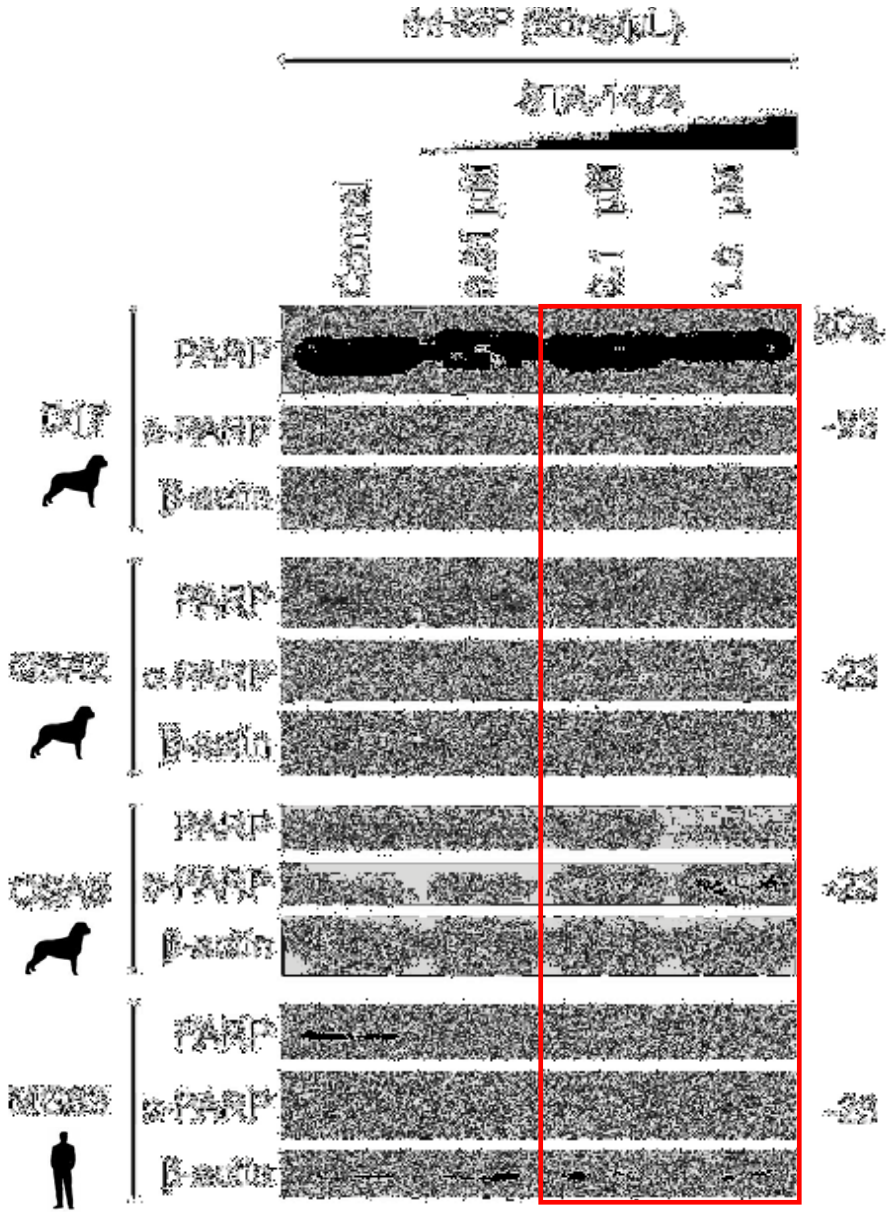


**p<0.001

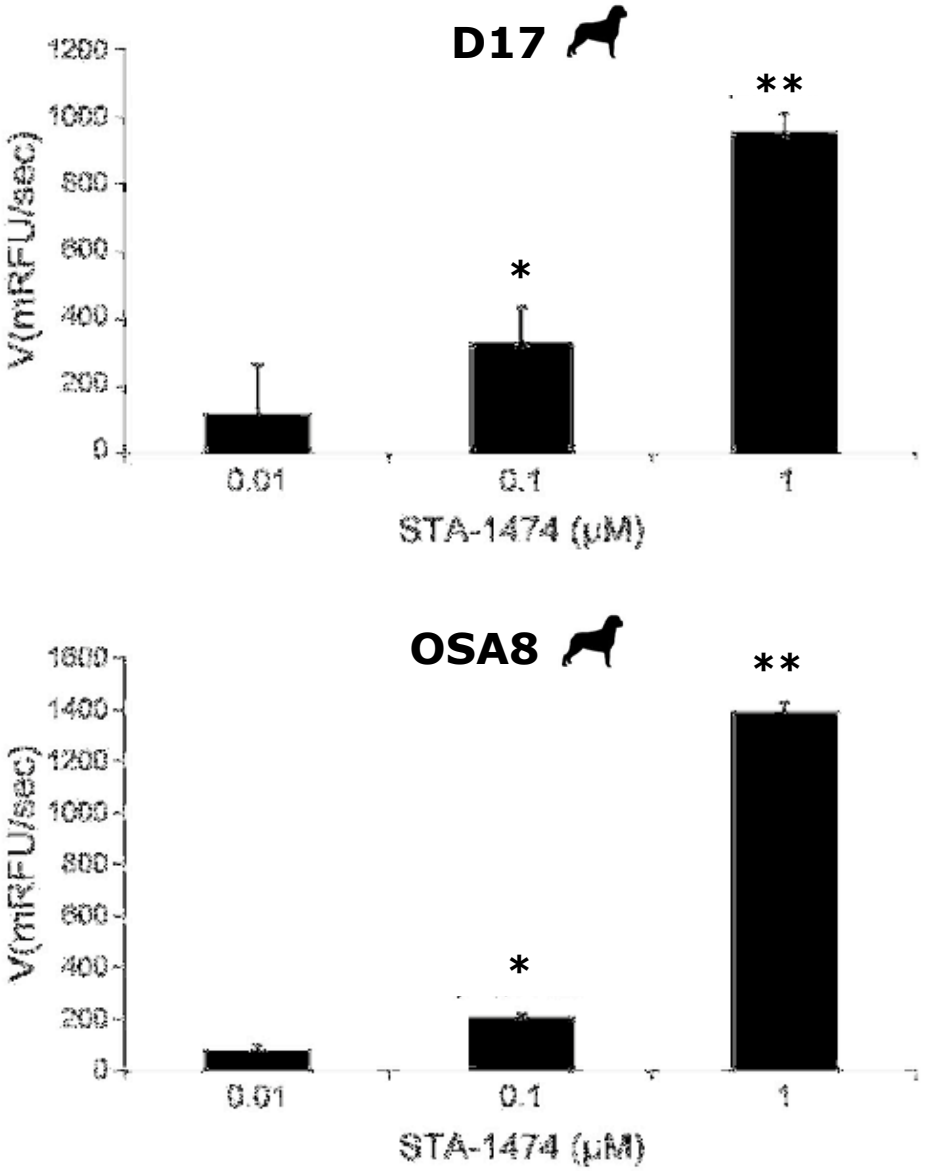
- **STA-1474 induces apoptosis in a dose-dependent manner in K9 OSA cells**
- **K9 OSA cells are more sensitive to STA-1474 treatment compared to normal K9 osteoblasts.**

STA-1474 induces caspase 3 activation

STA-1474 promotes a dose-dependent increase in PARP cleavage.



STA-1474 induces caspase 3/7 activation

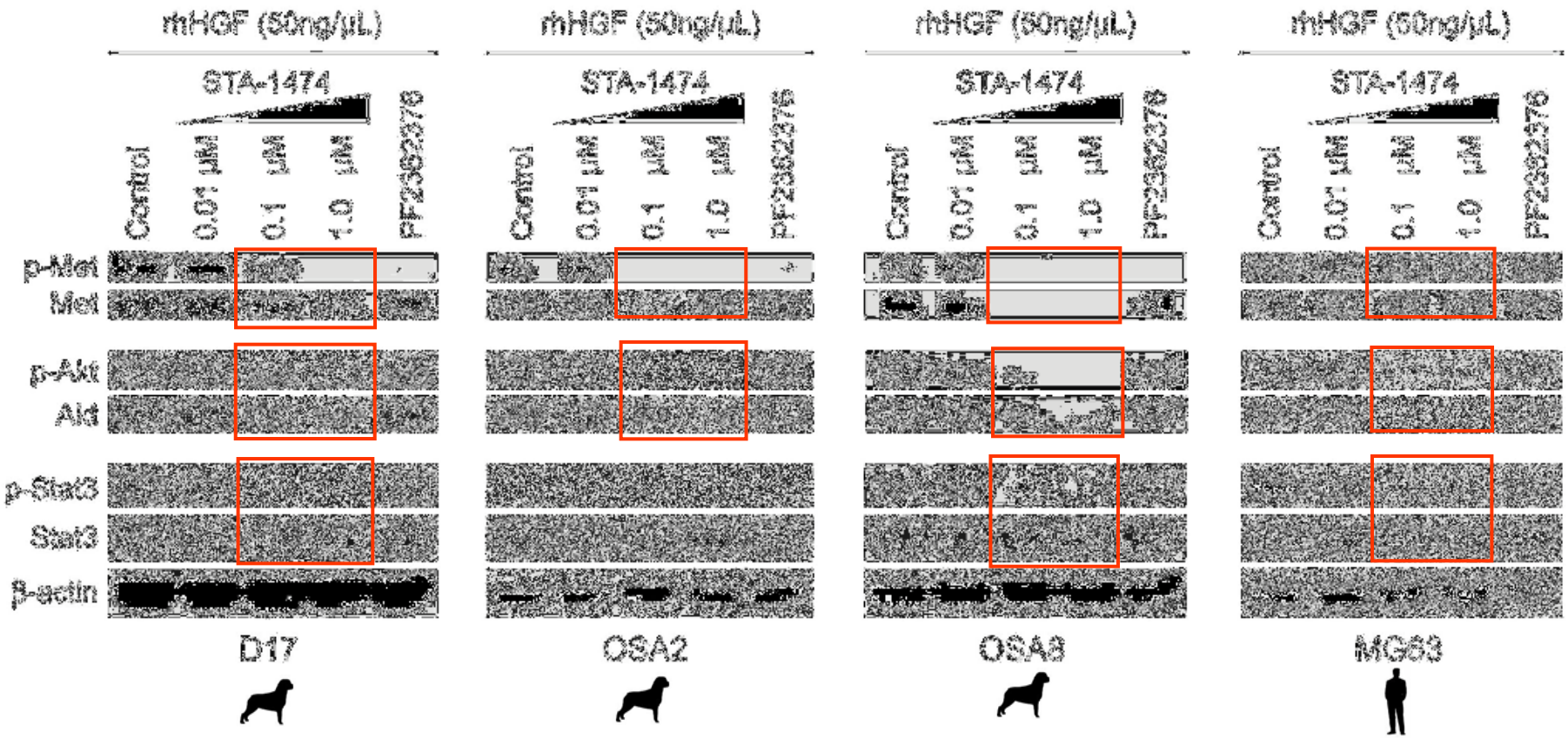


STA-1474 promotes a dose-dependent increase in caspase 3/7 activity.

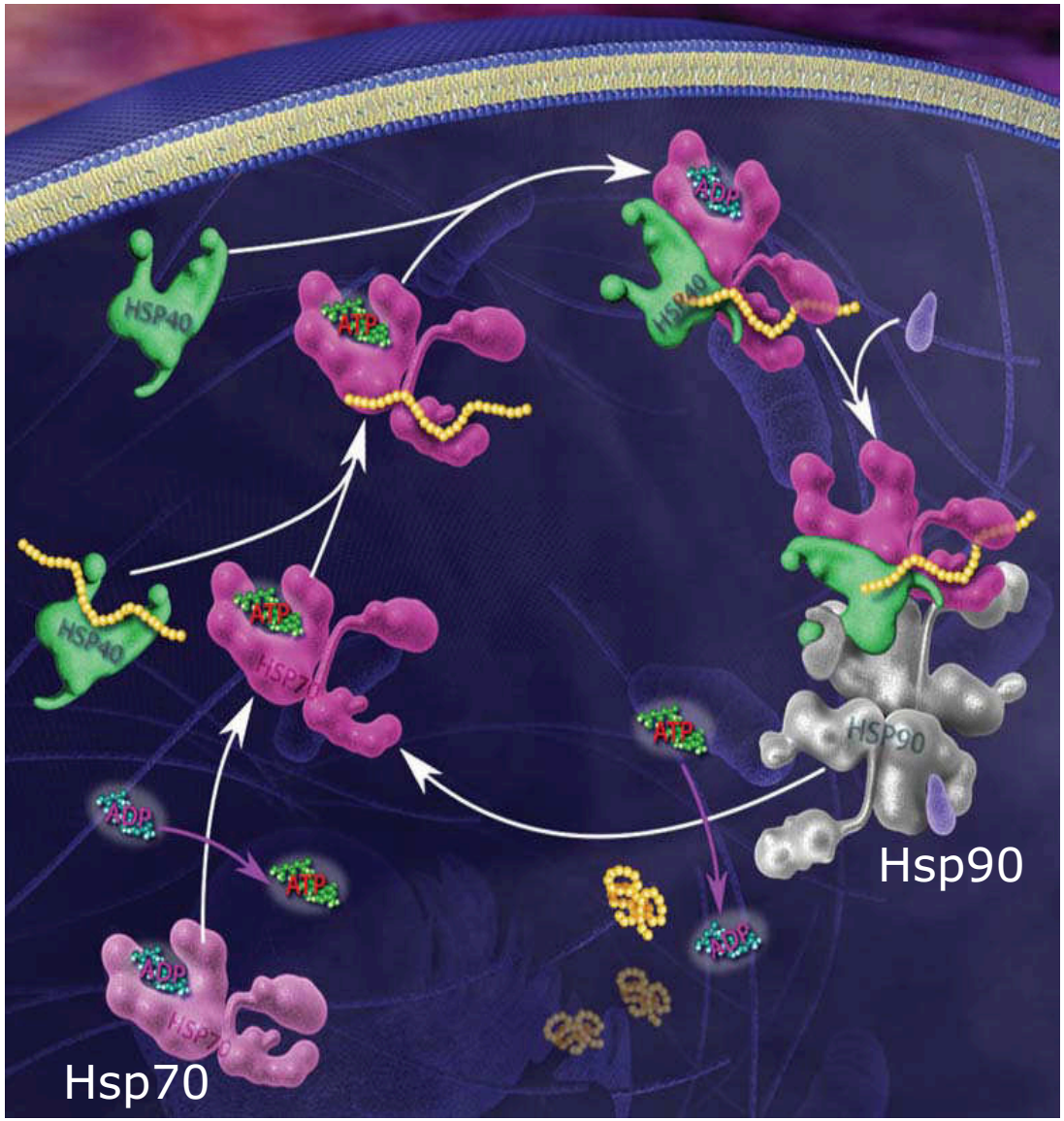
* p< 0.01

**p<0.001

STA-1474 down-regulates multiple client proteins in OSA



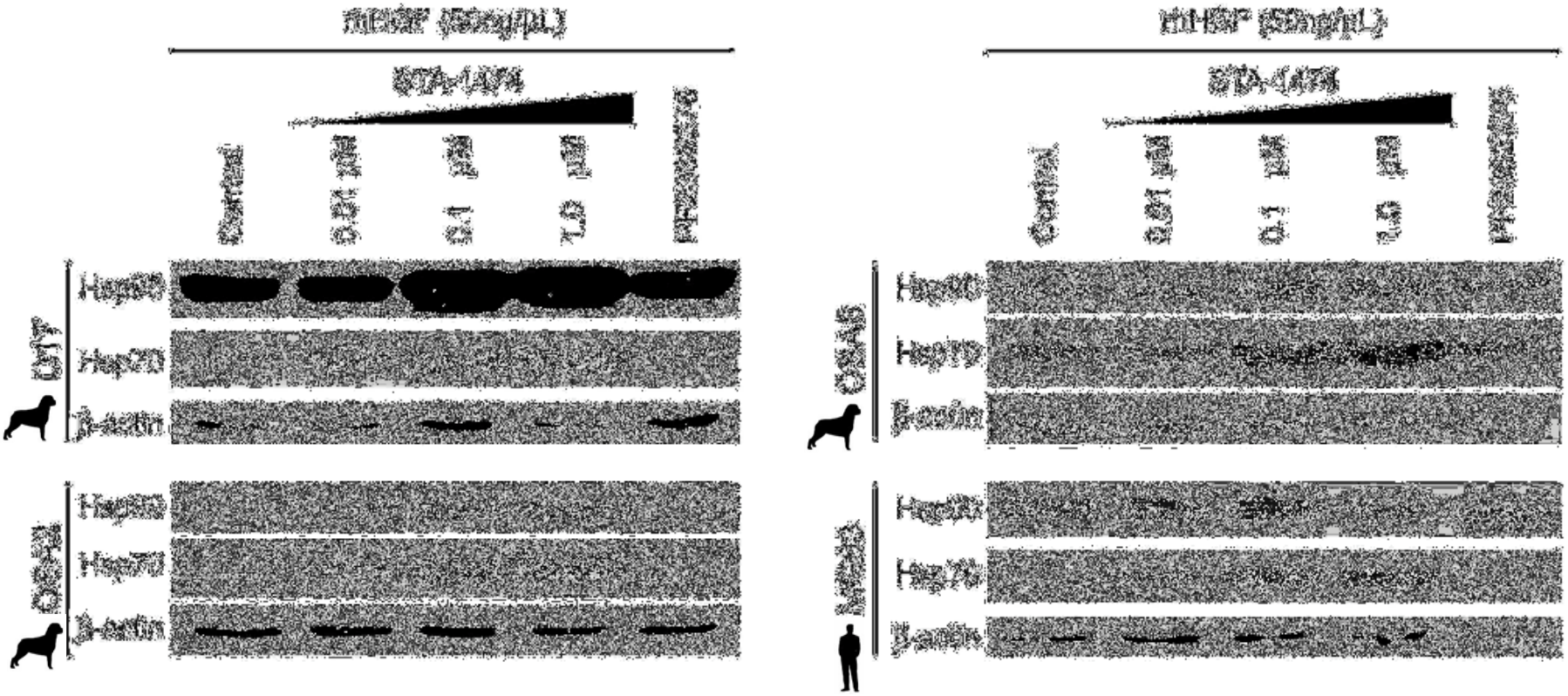
Induction of cellular stress



- Hsp70 is upregulated with cellular stress
- Used as a biomarker for Hsp90 inhibition

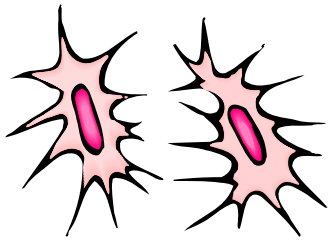
www.stressgen.com

STA-1474 up-regulates Hsp70



Hsp70 increases in a dose- dependent manner, consistent with a heat shock response induced by Hsp90 inhibition.

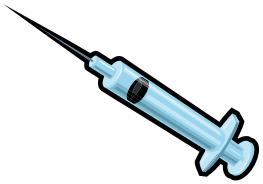
D17 OSA xenograft model



D17 cells (~0.4-1 x 10^7) injected into the flanks of female 7-8 week old SCID mice.

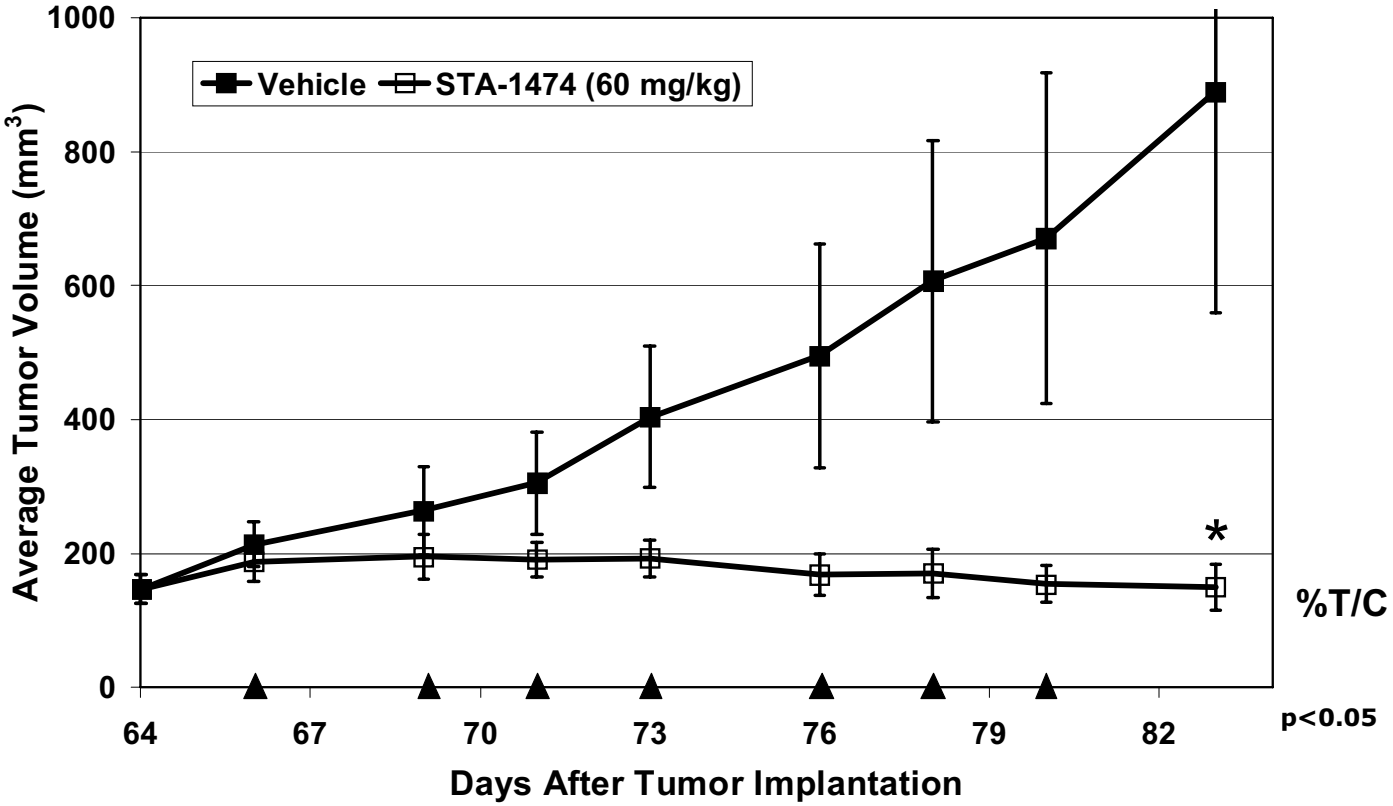
Mice randomized into treatment groups with avg tumor volumes/grp= ~150 mm^3.

IV bolus tail vein injection at 10 mL/kg with STA-1474 formulated in 10/18 DRD (10% DMSO, 18% Cremophor RH 40, 3.6% dextrose and 68.4% water).



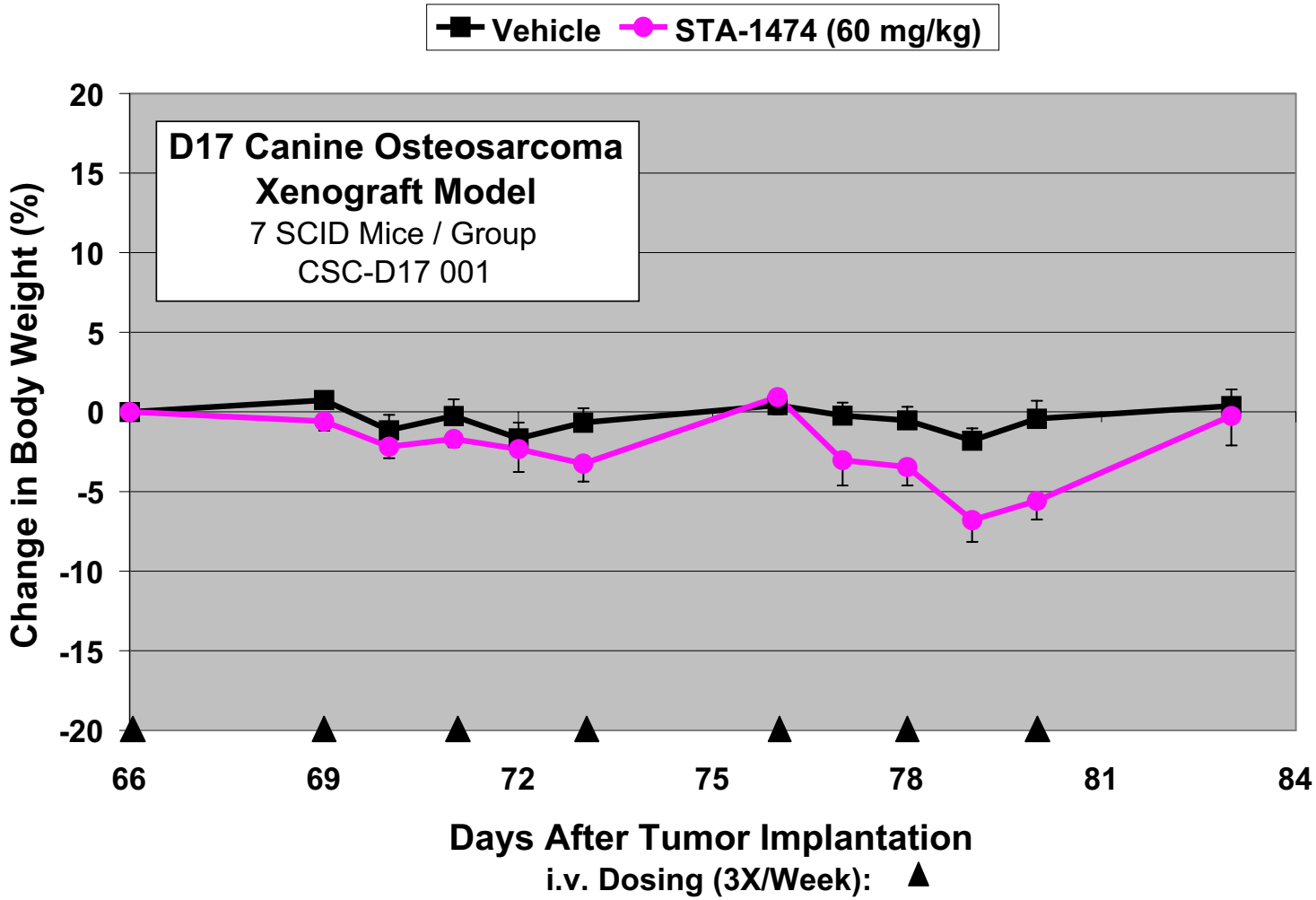
<u>Measurement of *in vivo* efficacy</u>
Change in avg tumor volume for STA-1474 treated group relative to the vehicle group.
<u>Tolerability</u>

STA-1474 inhibits tumor growth in an OSA xenograft model



- 60 mg/kg STA-1474 dosed 3x/wk for 2 wk significantly inhibited tumor growth with 57% of tumors regressing.
- Change in avg tumor volumes for STA-1474 treated group relative to vehicle group (%T/C= -6) indicated substantial efficacy.

Tolerability of STA-1474 in D17 xenograft model



Evaluation of STA-1474 mediated biologic effects *in vivo*

- D17 xenografts were allowed to grow to 150-200mm^3.
- Treated once with vehicle or 60 mg/kg STA-1474.
- Tumors harvested 72 h post treatment

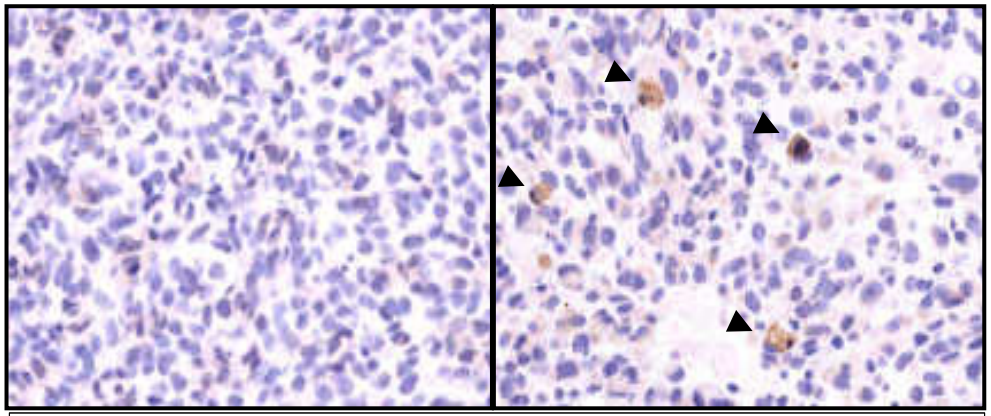
- Half flash frozen in liquid nitrogen for preparation of protein lysates
  - Immunoprecipitation/WB

- Half fixed in 10% neutral buffered formalin for IHC.
  - Cleaved caspase-3 (Apoptosis)

STA-1474 promotes apoptosis in an OSA xenograft model

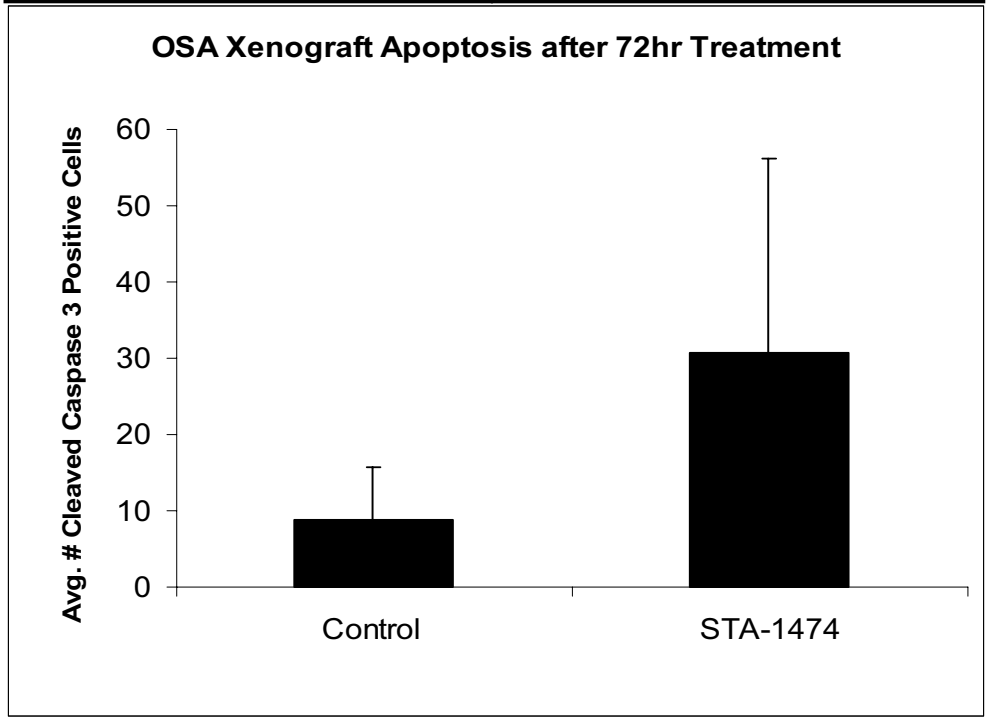
Cleaved Caspase-3

Control, 400X.



Cleaved Caspase-3

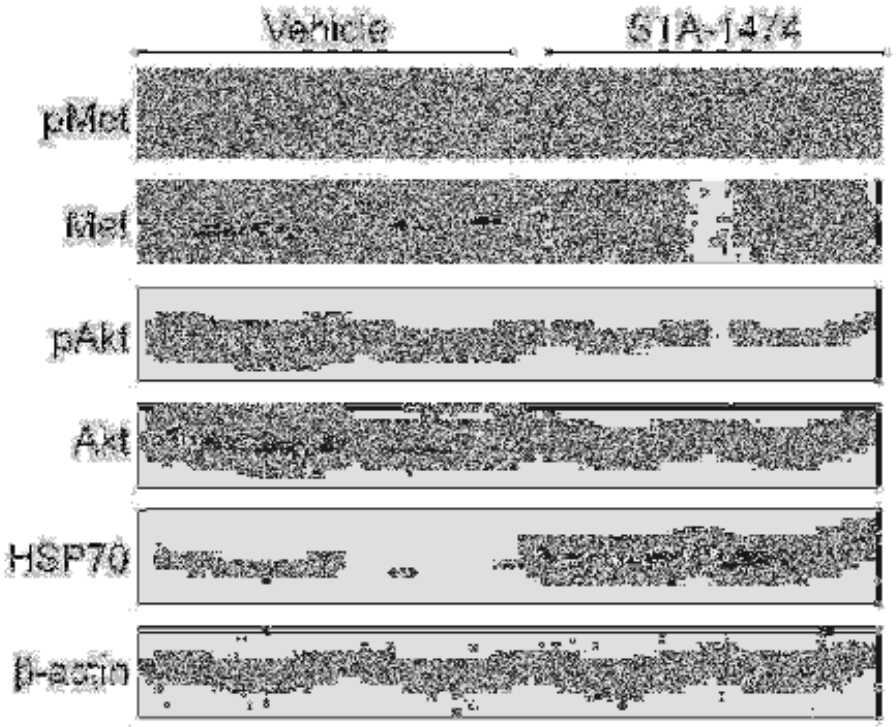
72h STA-1474, 400X.



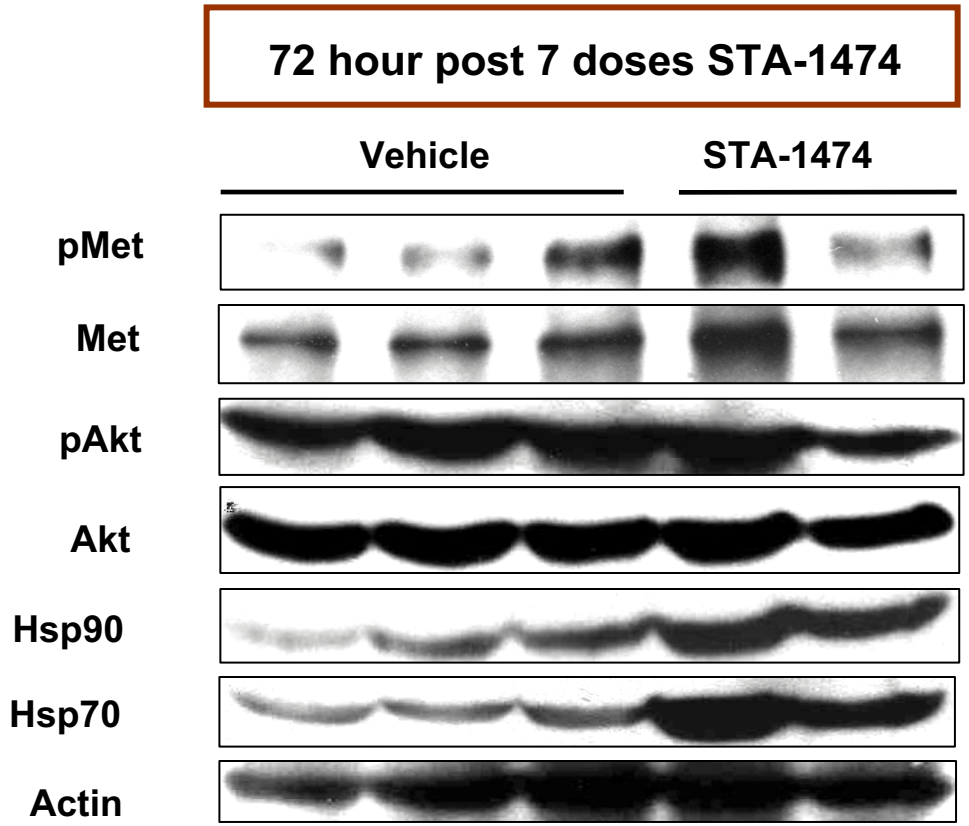
p=0.0407

STA-1474 down-regulates multiple clients in an OSA xenograft model

72 hrs post single dose of STA-1474



OSA Xenograft resistance to Hsp90 inhibition



Summary

- STA-1474 is a potent inhibitor of cell proliferation in multiple OSA cell lines (canine and human) and promotes cell death via caspase 3/7 mediated apoptosis.
- STA-1474 shows selectivity for malignant OSA cells versus non-malignant cells mediated by Hsp90 co-chaperone association.
- STA-1474 treatment induces Hsp70 upregulation, serving as a potential biomarker for Hsp90 inhibition in OSA cells.

Summary

- STA-1474 targets multiple signal transduction pathways in canine and human OSA cell lines, down-regulating p-Met, Met, p-Akt, and Akt both *in vitro* and *in vivo*. Total Stat3 levels remained unchanged.

- These data support the role of Hsp90 as a relevant target for therapeutic intervention in OSA.

Significance

- There are no consistently effective therapeutic strategies to treat metastatic OSA in dogs and little progress has been made to improve survival rates over the last decade.

- STA-1474 is a promising therapy for OSA
  - STA-1474 treatment selective for neoplastic vs. normal tissue

Acknowledgements

- **Advisor**: Dr. Cheryl London
- **Committee**:
  - Dr. William Kisseberth
  - Dr. Laura Rush
  - Dr. Brett Hall
- **Lab Members**:
  - Dr. Cindy Lin
  - Dr. Stacey Fossey
  - Dr. Melanie McMahon
  - Misty Bear
- **Synta:**
  - Jim Barsoum
  - Weiwin Ying
  - Kevin Foley
  - Robert Mihalek

- **Funding**: Morris Animal Foundation, Synta Pharmaceuticals