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

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Heat shock proteins

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

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 \textit{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
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

- 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.
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).

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

**T tolerability**
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

D17 Canine Osteosarcoma
Xenograft Model
7 SCID Mice / Group
CSC-D17 001

Change in Body Weight (%)

Days After Tumor Implantation
i.v. Dosing (3X/Week): ▲
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.

OSA Xenograft Apoptosis after 72hr Treatment

Avg. # Cleaved Caspase 3 Positive Cells
0 10 20 30 40 50 60
Control STA-1474

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

72 hour post 7 doses STA-1474
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
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