The Next Generation Hsp90 Inhibitor STA-9090, Currently in Phase 2 Trials, Displays Potent *in vitro* and *in vivo* Activity

David A. Proia, Suqin He, Jane Kepros, Tim Korbut, Luisa Shin Ogawa, Jim Sang, Donald Smith, Chaohua Zhang, Ronald K. Blackman, Kevin P. Foley, Takayo Inoue, Weiwen Ying and Keizo Koya

Synta Pharmaceuticals Corp., Lexington, Massachusetts USA

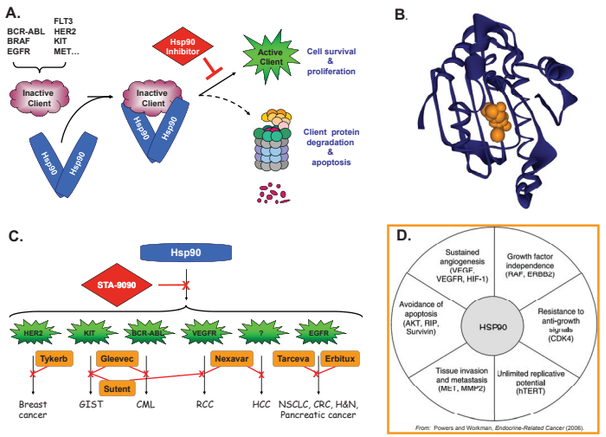
Abstract (#C05)

Background: Heat shock protein 90 (Hsp90) is a molecular chaperone that mediates the post-translational folding of its protein substrates ("client proteins"). Many client proteins play critical oncogenic or proliferative roles, making cancer cells especially sensitive to Hsp90 inhibition. Here we report on the characterization of STA-9090, a next-generation Hsp90 inhibitor that is structurally unrelated to and substantially more potent than the first-generation ansamycin Hsp90 inhibitors, such as 17-AAG and IPI-504. STA-9090 is currently in multiple Phase 1 and Phase 2 clinical trials in patients with hematologic and solid tumors, including Phase 2 trials in non-small cell lung cancer (NSCLC) and gastrointestinal stromal tumor (GIST).

Results: In *in vitro* studies, treatment with STA-9090 induces the rapid and sustained degradation of known oncogenic Hsp90 client proteins such as mutant EGFR, HER2, KIT and JAK2. Growth inhibition (IC_{50}) of cell lines typically occurs in the range of 1 to 100 nM (median = 13 nM) for the ~60 hematological and solid tumor cell lines tested. On average, it is ~30 times more potent than 17-AAG. Importantly, STA-9090 also retains its potency against cell lines expressing mutated kinases that confer resistance to kinase inhibitors such as erlotinib and imatinib. In mouse studies, STA-9090 demonstrates single-agent activity in a wide variety of human tumor cell line subcutaneous xenograft models, including those representing both solid tumor and hematological malignancies. To investigate the potential for therapeutic synergy, we also examined combinations of STA-9090 with a taxane, either paclitaxel or docetaxel. Both taxanes showed strong synergy with STA-9090 in *in vitro* studies. Similarly, in mouse xenograft models, greater efficacy was seen with the combinations than any of the single agents alone. The combination treatment did not result in additional toxicity.

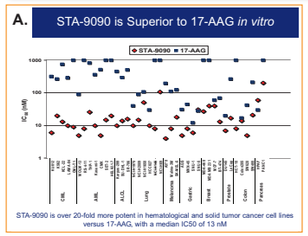
Conclusions: STA-9090 is a highly potent Hsp90 inhibitor that *in vitro* and *in vivo* rapidly leads to the degradation of Hsp90 client proteins and induces apoptosis in a broad range of cancer types, including those resistant to targeted kinase inhibitors. Taken together, these results suggest that further exploration of STA-9090 both as a single agent and in combination with taxanes in the clinic is warranted.

Introduction

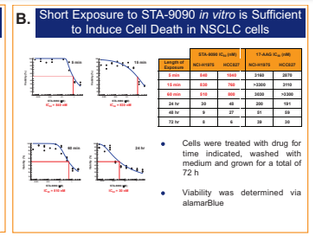


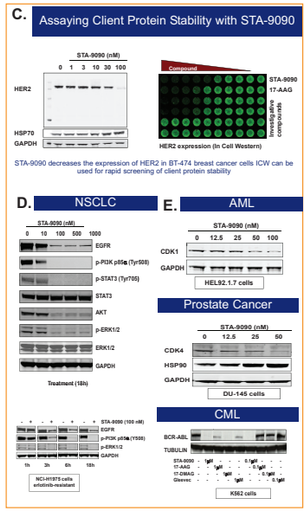
STA-9090: A multifunctional drug for the treatment of cancer. (A) The molecular chaperone Hsp90 plays a key role in regulating the correct folding, stability and activity of many important signal transduction molecules that have been implicated in the pathophysiology of cancer. For this reason, Hsp90 has been considered to be an attractive target for the development of novel cancer therapies. Inhibition of Hsp90 by STA-9090 (1, 2) induces the proteasome-mediated degradation of these so-called "client proteins", resulting in cell cycle arrest and apoptosis of cancer cells. (B) Hsp90:STA-9090 X-ray co-crystal structure. STA-9090 competes with ATP for binding to the N-terminal domain of Hsp90. (C) STA-9090 treatment concomitantly affects multiple clinically validated drug targets. Given the diversity of Hsp90 client proteins that have been identified, STA-9090 has the potential to show anti-cancer activity against a wide variety of different human tumor types. Additionally, STA-9090 may provide a means to simultaneously target multiple pathways in a single cancer type, thereby potentially bypassing the redundancies in regulatory pathways and mechanisms of resistance that are commonly found in cancer cells. (D) STA-9090 is a multifunctional molecule that can target multiple critical signaling pathways in cancer cells.

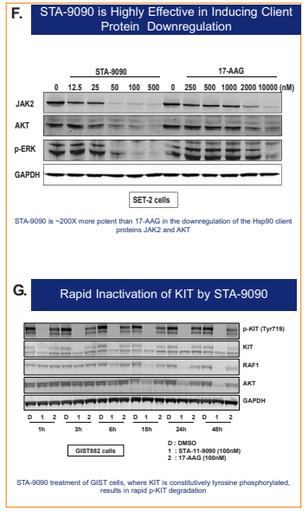
STA-9090 is Highly Potent In a Wide Variety of Tumors Cells



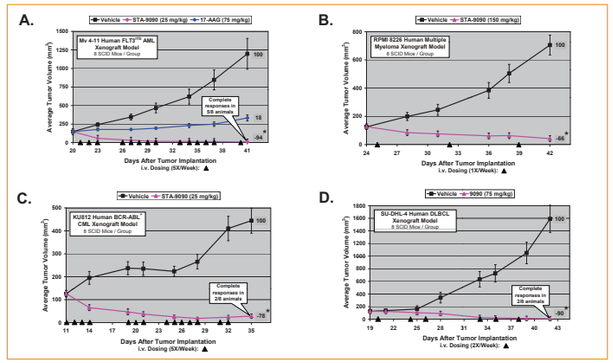
STA-9090 is over 20-fold more potent in hematological and solid tumor cancer cell lines versus 17-AAG, with a median IC50 of 13 nM



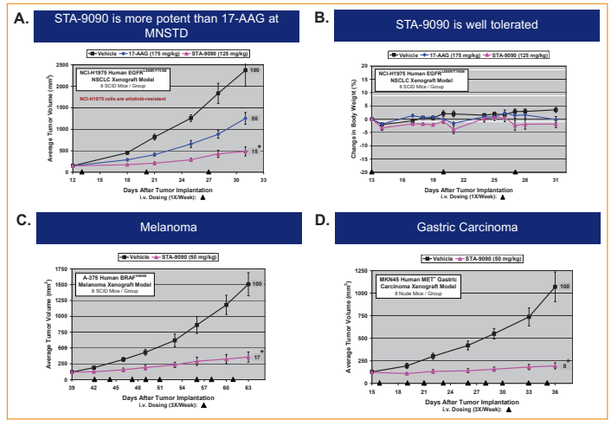




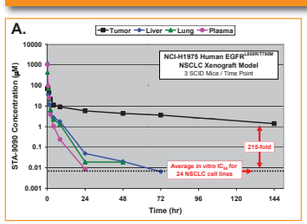
STA-9090 Has Potent *in vivo* Activity in Hematological Subcutaneous Xenograft Models



STA-9090 Has *in vivo* Activity in Solid Tumor Xenograft Models



STA-9090 Accumulates in Tumors Relative to Normal Tissues



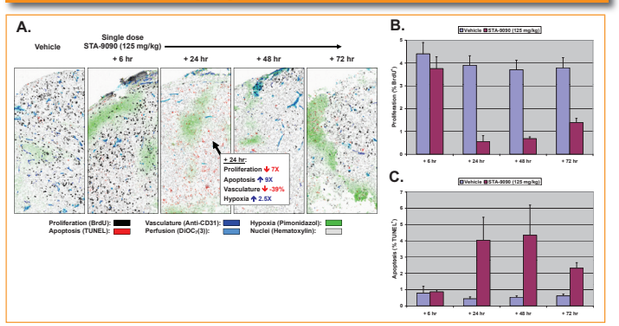
B.

Tissue	$t_{1/2}$ (h)	t_{max} (min)	C_{max} (μM)	AUC_{inf} (μMxh)	V_{ss} (L/kg)	CL (L/h/kg)
Plasma	3	5	1025	399	0.23	0.86
Tumor	58.3	30	74.8	809	--	--
Liver	5.6	5	1165	352	--	--
Lung	5.4	5	441	190	--	--

1 μM = 1 nmol/g of tissue

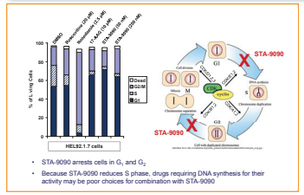
STA-9090 concentration in xenograft tumors remains above the *in vitro* IC50 for at least 6 days after a single drug dose

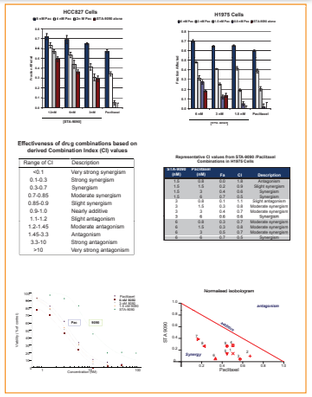
STA-9090 Treatment Results in Long-Lasting Inhibition of Proliferation and Induction of Apoptosis in Tumors



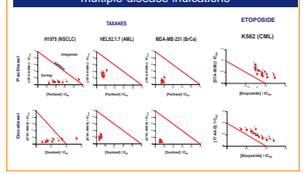
STA-9090 treatment in xenograft models results in decreased tumor proliferation and vasculature, resulting in apoptosis and hypoxia

Taxanes Synergize With STA-9090 in *in vitro* Studies



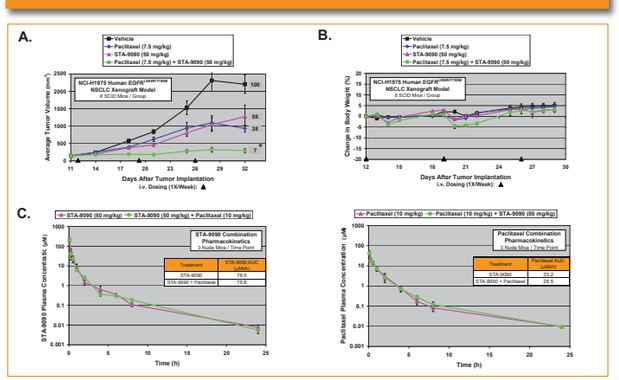


Taxanes, not S-phase inhibitors, synergize across multiple disease indications



In vitro, combinations of STA-9090 and taxanes near or below their respective IC_{50}'s are synergistic

Combination Efficacy *in vivo* of STA-9090 and Paclitaxel



Once weekly dosing of STA-9090 enhances paclitaxel efficacy without increasing systemic toxicity

Summary

- STA-9090 has potent *in vitro* and *in vivo* efficacy in a broad range of hematological and solid tumor cell lines
- STA-9090 demonstrates superiority to 17-AAG in preclinical studies and rapidly leads to the degradation of client proteins critical for oncogenic survival and proliferation
- In mouse models involving tumors resistant to targeted kinase inhibitors, STA-9090 treatment significantly increased survivability
- Combinations of STA-9090 and taxanes showed synergy both *in vitro* and *in vivo* and showed no PK interactions
- STA-9090 is currently being examined in multiple Phase 1 and Phase 2 clinical trials in subjects with solid tumor or hematological malignancies

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For further information on STA-9090: www.syntapharma.com