

Synergy Between the Novel Hsp90 Inhibitor STA-9090 and Taxanes in Preclinical Models of NSCLC

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Abstract

The molecular chaperone heat shock protein 90 (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 non-small cell lung cancer (NSCLC), including: BRAF, EGFR, HER2, MET and VEGFR. STA-9090 is a novel small molecule Hsp90 inhibitor that is structurally unrelated to the first-generation ansamycin Hsp90 inhibitors 17-AAG and IPI-504. STA-9090 competes with ATP for binding to the N-terminal domain of Hsp90, thereby inducing proteasome-mediated degradation of Hsp90 client proteins preferentially in cancer versus normal cells. STA-9090 is currently being evaluated in multiple Phase 1 and Phase 2 clinical trials in solid tumor and hematological malignancies, as well as in an open-label, multi-center Phase 2 clinical trial in patients with advanced NSCLC that harbor wild type or activated forms of EGFR and KRAS. We have investigated the activity of STA-9090 alone and in combination with taxanes in preclinical NSCLC models.

In vitro, STA-9090 potently inhibited cell proliferation in 24/24 human NSCLC cell lines (median IC₅₀ = 6.5 nM; range 1-31 nM), irrespective of EGFR, HER2 or KRAS mutational status. STA-9090 was also active against cell lines that displayed resistance to erlotinib and/or 17-AAG. *In vivo*, intravenous treatment with STA-9090 at 80-100% of its highest non-severely toxic dose induced stable disease in both the HCC827 (erlotinib-sensitive EGFR^{del746-750}) and NCI-H1975 (erlotinib-resistant EGFR^{L858R/T790M}) xenograft models in mice. In the HCC827 model, tumor progression could be inhibited for at least 7 weeks using a one time per week dosing schedule. Further, in an inducible bitransgenic mouse model in which expression of mutant HER2^{YVMA} resulted in the development of adenocarcinoma lung tumors, 3/4 animals treated with STA-9090 displayed partial responses as measured by MRI. This result correlated with decreased HER2 expression in tumors.

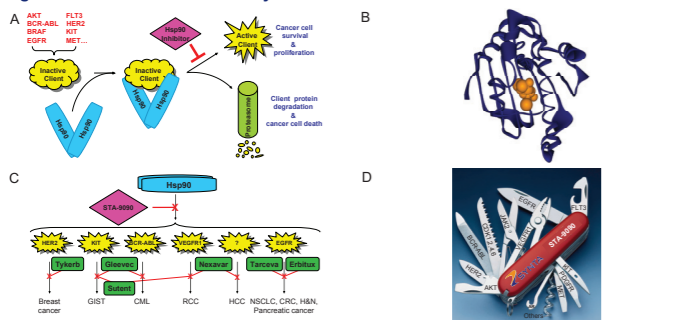
In the NCI-H1975 model, STA-9090 displayed superior efficacy to 17-AAG, and inhibition of tumor growth was correlated with decreased expression of EGFR and other Hsp90 client proteins. Importantly, these effects persisted in tumors for up to 3-6 days after treatment. Similarly, histological analysis of tumors indicated that STA-9090 inhibited cell proliferation by 7-fold and induced apoptosis by 9-fold, with maximal effects being observed at 1-3 days after treatment. Consistent with these observations, STA-9090 accumulated in tumors with a half-life of 58 hr versus 3-6 hr in normal tissues and plasma, and the tumor concentration remained 215-fold higher than the average *in vitro* IC₅₀ at 6 days after a single drug dose.

The taxanes paclitaxel and docetaxel are widely used in the treatment of advanced-stage NSCLC. To examine the combination of STA-9090 plus taxanes, *in vitro* studies were conducted with NCI-H1975 cells. Using the median-effect method of Chau and Talalay, STA-9090 in combination with either paclitaxel or docetaxel displayed combination index values within the synergistic range (0.23-0.65 CI). *In vivo*, in the NCI-H1975 xenograft model, the combination of STA-9090 and paclitaxel displayed greater efficacy than either single agent alone (%T/C values of 7, 38 and 55, for the combination, paclitaxel and STA-9090, respectively). Synergy between STA-9090 and paclitaxel was not due to alterations in the pharmacokinetics of either agent, and the combination treatment did not result in additional toxicity.

Our results demonstrate that STA-9090 is a highly potent Hsp90 inhibitor that displays broad *in vitro* and *in vivo* anti-cancer activity in preclinical models of NSCLC. STA-9090 also synergizes with paclitaxel and docetaxel. Taken together, these results suggest that further exploration of STA-9090 as both a single agent and in combination with taxanes in NSCLC patients is warranted.

Introduction

Figure 1. STA-9090: A Swiss Army knife 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 (orange) competes with ATP for binding to the N-terminal domain of Hsp90 (blue). (C) STA-9090 treatment concomitantly affects multiple clinically validated drug targets. Given the diversity of Hsp90 client proteins that have been identified, STA-9090 is expected to show anti-cancer activity against a wide variety of different human tumor types. Additionally, STA-9090 should 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 can be thought of as a "Swiss Army knife" that can target multiple critical signaling pathways in cancer cells.

Table 1. STA-9090 displays superior *in vitro* cytotoxicity relative to 17-AAG against erlotinib-sensitive and -resistant NSCLC cell lines

Cell Line	EGFR	HER2	KRAS	Other	Erlotinib	17-AAG IC ₅₀ *	STA-9090 IC ₅₀ *
H3255	L858R	Wild-type	Wild-type		Sensitive	58	22
HCC827	Del E746_A750	Wild-type	Wild-type		Sensitive	18	7
PC9	Del E746_A750	Wild-type	Wild-type		Sensitive	7	2
HCC4006	Del L747_E749_A750P	Wild-type	Wild-type		Sensitive	25	12
HCC2279	Del E746_A750	Wild-type	Wild-type		Medium	>10000	31
NCI-H1975	L858R/T790M	Wild-type	Wild-type		Resistant	75	<1
NCI-H820	Del E746_L751_Ins I7790M	Wild-type	Wild-type		Resistant	34	<1
DFCI-LU011	Del L747_E749_A750P	Wild-type	Wild-type		Resistant	111	2
NCI-H1650	Del E746_A750	Wild-type	Wild-type		Resistant	7	7
NCI-H1781	G776insV_G/C	Wild-type	Wild-type		Resistant	22	2
NCI-H1734	Wild-type	Wild-type	G13C		Resistant	96	12
AS49	Wild-type	Wild-type	G12S		Resistant	75	22
NCI-H460	Wild-type	Wild-type	G61H		Resistant	77	14
NCI-H358	Wild-type	Wild-type	G12C		Resistant**	3	1
A427	Wild-type	Wild-type	G12D		Resistant	4	<1
NCI-H441	Wild-type	Wild-type	G12V		Resistant	111	26
NCI-H1299	Wild-type	Wild-type	NRAS (G61K)		Resistant	36	6
NCI-H1666	Wild-type	Wild-type	BRAF (G466V)		Medium	27	6
NCI-H1819	Wild-type (Amp)	Wild-type	PIK3CA		Resistant	749	7
NCI-H1703	Wild-type	Wild-type	PDGFRA (Amp)		Resistant	3	3
NCI-H596	Wild-type	Wild-type	RB Null		Resistant	3,500	7
NCI-H522	Wild-type	Wild-type	Wild-type		Resistant	7	6
HCC1833	Wild-type	Wild-type	Wild-type		Resistant	4	<1
Calu-3	Wild-type (Amp)	Wild-type (Amp)	Wild-type		Resistant**	16	9

A panel of human NSCLC cell lines was treated with STA-9090 or 17-AAG for 72 hr and cell viability was measured by MTT assay. IC₅₀ values were determined using Xlfit. STA-9090 displayed potent activity against all 24 cell lines, irrespective of EGFR, HER2 or KRAS mutational status. The median IC₅₀ values for STA-9090 and 17-AAG were 6.5 nM and 30.5 nM, respectively. STA-9090 was also active against cell lines that displayed resistance to erlotinib and/or 17-AAG.

Figure 2. STA-9090 is highly efficacious *in vivo* in both erlotinib-sensitive and -resistant NSCLC xenograft models and displays superior activity relative to 17-AAG

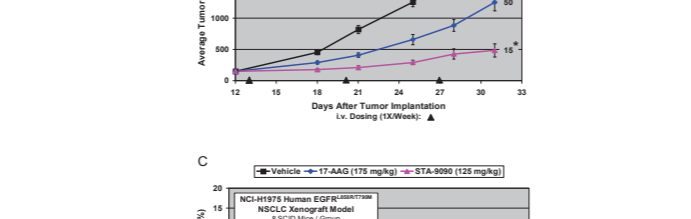
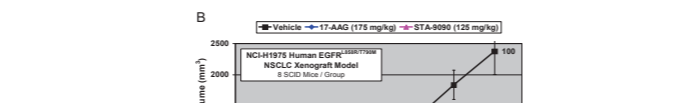
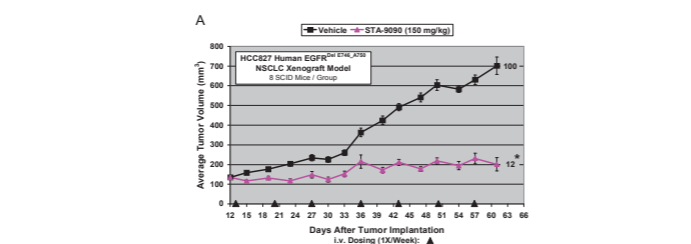
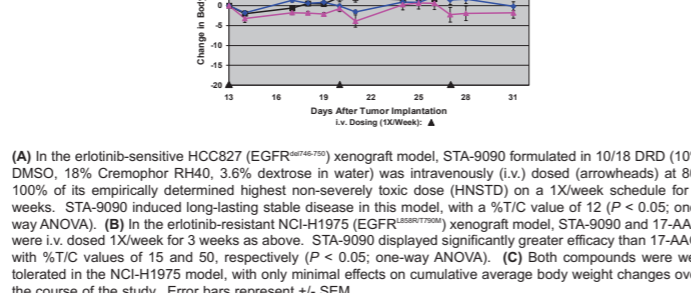
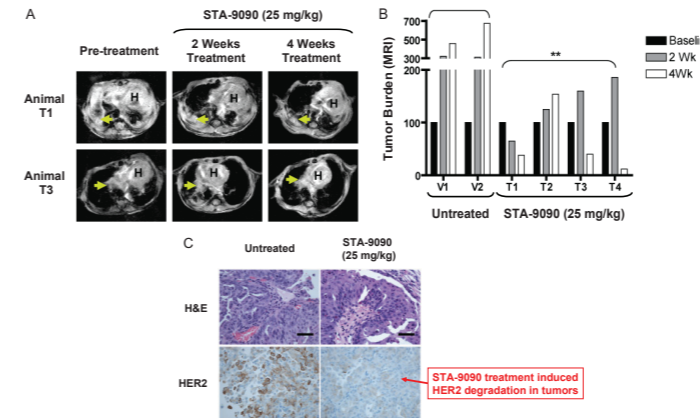


Figure 5. STA-9090 accumulates in tumors relative to normal tissues



Results

Figure 3. STA-9090 is highly efficacious in a *de novo* bitransgenic mouse lung cancer model expressing activated HER2^{YVMA}



The *in vivo* efficacy of STA-9090 was examined in an inducible bitransgenic mouse lung cancer model in which HER2^{YVMA} (encoding HER2^{YVMA}, the most common mutationally activated form of HER2 occurring in human NSCLC) was specifically expressed in lung type II pneumocytes, leading to the development of invasive adenocarcinoma (3). Bitransgenic animals were treated for 4 weeks with doxycycline to induce HER2^{YVMA} expression, imaged by MRI to confirm tumor burden, and then i.v. dosed with either 25 mg/kg STA-9090 ($N = 4$; T1-4) or 10/18 DRD vehicle ($N = 2$; V1-2) on a 3X/week schedule for 4 weeks. (A) Representative MRI scans performed after 2 or 4 weeks of STA-9090 treatment displayed substantial tumor regression (arrowheads). Position of heart is indicated (H). (B) Quantitative MRI analysis demonstrated that tumor regression occurred in 3/4 STA-9090-treated animals after 4 weeks of treatment, whereas substantial tumor progression was observed in vehicle-treated animals ($P < 0.001$). (C) Representative anti-HER2 immunohistochemistry performed on peripheral lung tumors after 3 doses of STA-9090 (400X; 50 μM scale bar). Treatment with STA-9090 resulted in dramatically decreased HER2 expression.

Figure 4. STA-9090 treatment results in long-lasting downregulation of Hsp90 client proteins, inhibition of proliferation and induction of apoptosis in xenograft tumors

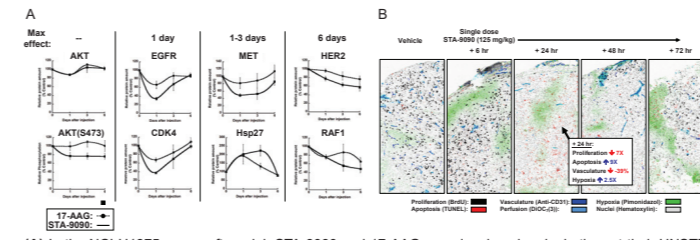


Figure 5. STA-9090 accumulates in tumors relative to normal tissues

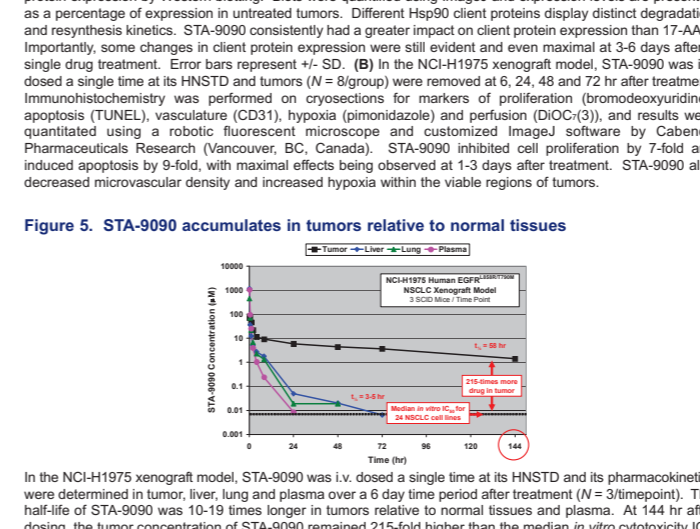


Figure 6. *In vitro* synergy between STA-9090 and paclitaxel or docetaxel

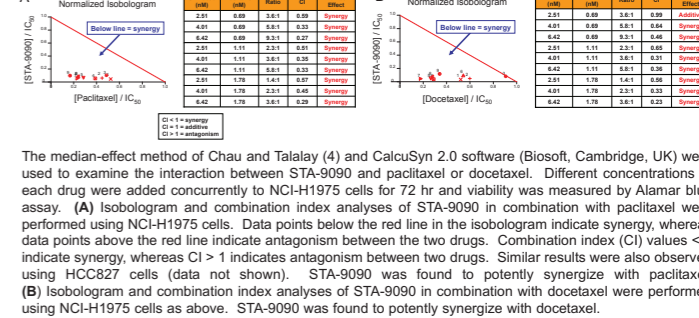
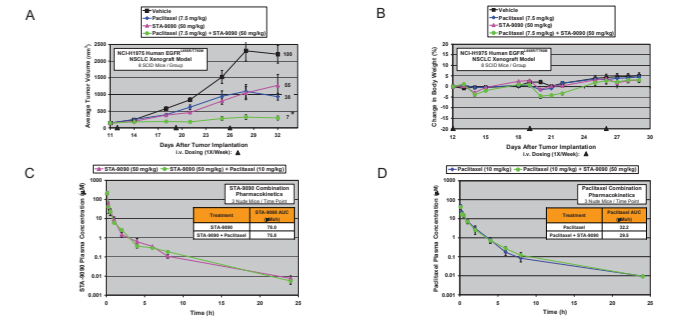


Figure 7. *In vivo* synergy between STA-9090 and paclitaxel in an erlotinib-resistant NSCLC xenograft model



(A) In the erlotinib-resistant NCI-H1975 (EGFR^{L858R/T790M}) xenograft model in C.B-17 SCID mice, sub-HNSTDs of STA-9090 and paclitaxel (formulated in 10/18 DRD) were i.v. dosed as single agents or concurrently on a 1X/week schedule for 3 weeks. The combination of STA-9090 and paclitaxel displayed significantly greater efficacy than either single agent alone, with %T/C values of 7, 38 and 55, for the combination, paclitaxel and STA-9090, respectively ($P < 0.05$; one-way ANOVA). Similar results were also observed in the HCC827 model (data not shown). (B) Combination treatment did not result in additional toxicity relative to the single agents, with only minimal effects on cumulative average body weight changes over the course of the study. (C) Paclitaxel did not affect the plasma exposure of STA-9090 in CD-1 Nude mice. (D) STA-9090 did not affect the plasma exposure of paclitaxel in CD-1 Nude mice. Error bars represent +/- SEM.

Conclusions

- STA-9090 is a novel small molecule Hsp90 inhibitor that is structurally unrelated to the first-generation ansamycin Hsp90 inhibitors 17-AAG and IPI-504.
- STA-9090 was ~4.7-fold more potent than 17-AAG on a panel of NSCLC cell lines.
- STA-9090 retained full potency against both erlotinib- and 17-AAG-resistant NSCLC cell lines, irrespective of EGFR, HER2 or KRAS mutational status.
- STA-9090 was highly efficacious in both erlotinib-sensitive and -resistant lung cancer models in mice. STA-9090 specifically accumulated in tumors and induced long-lasting effects on Hsp90 client protein expression, tumor cell proliferation and apoptosis.
- STA-9090 dramatically enhanced the activity of taxanes in an erlotinib-resistant NSCLC model.
- STA-9090 is currently being examined in multiple Phase 1 and Phase 2 clinical trials, including an open-label, multi-center Phase 2 clinical trial in patients with advanced NSCLC that harbor wild type or activated forms of EGFR and KRAS.

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