

# Pharmacodynamic Analysis of the Hsp90 Inhibitor STA-9090 in a Lung Cancer Xenograft Model Supports an Infrequent Dosing Schedule in the Clinic

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## Abstract (#C91)

**Background:** Heat shock protein 90 (Hsp90) is a molecular chaperone that is required for the stability and function of many important signal transduction proteins that regulate the growth of cancer cells. Hsp90 inhibition results in ubiquitination and proteasomal degradation of these client proteins, which include clinically validated drug targets such as BCR-ABL, mutant EGFR, HER2, KIT and VEGFR. STA-9090 is a novel small molecule Hsp90 inhibitor that is currently in multiple Phase 1/2 clinical trials in solid tumor and hematological malignancies. STA-9090 is structurally unrelated to the first-generation ansamycin Hsp90 inhibitors 17-AAG and IPI-504 and inhibits Hsp90 by binding to its N-terminal ATP-binding pocket (1, 2).

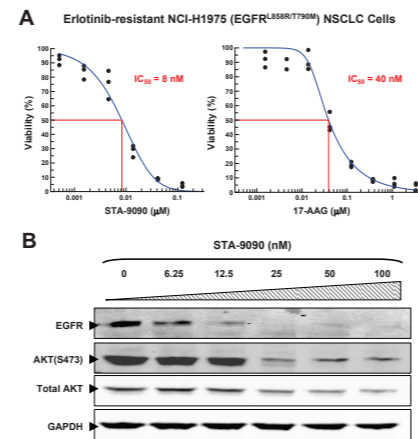
Although Hsp90 inhibitors such as STA-9090 induce rapid client protein degradation, cell cycle arrest and apoptosis of cancer cells, it is possible that frequent drug dosing in the clinic may be needed to continuously maintain decreased client protein expression and avoid renewed tumor growth. To investigate this possibility, we conducted *in vitro* and *in vivo* studies using the human NCI-H1975 non-small cell lung cancer (NSCLC) cell line, which expresses the Hsp90 client protein EGFR<sup>L858R/T790M</sup>, a mutationally activated and erlotinib-resistant form of the epidermal growth factor receptor.

**Results:** In an *in vitro* cytotoxicity assay using this cell line, STA-9090 and 17-AAG displayed IC<sub>50</sub> values of 8 and 40 nM after 72 hr drug exposure, respectively. These results closely correlated with decreased expression of EGFR<sup>L858R/T790M</sup> and other Hsp90 client proteins such as phosphorylated (S473) and total AKT. Unexpectedly, exposure to STA-9090 for only 1 hr still resulted in an IC<sub>50</sub> of 510 nM, suggesting that even brief drug exposure *in vivo* may be sufficient to affect tumor growth.

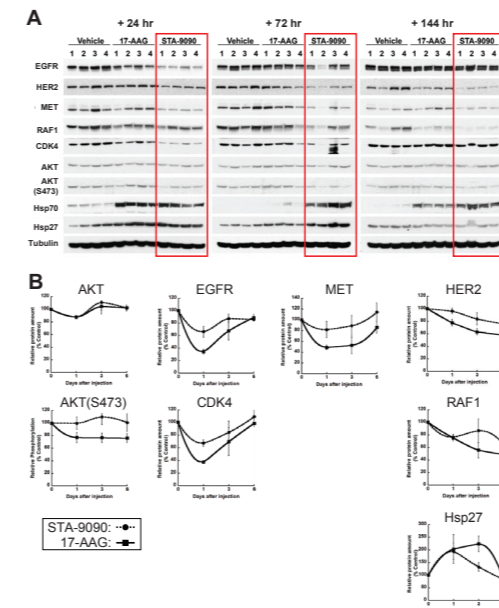
Consistent with this, intravenous dosing of 125 mg/kg STA-9090 on a 1X/week x 3 week schedule (~80-100% of the highest non-severely toxic dose) induced stable disease in the NCI-H1975 xenograft model, whereas 175 mg/kg 17-AAG resulted in progressive disease, with %T/C values of 15 and 50, respectively. Inhibition of tumor growth was correlated with decreased expression of EGFR<sup>L858R/T790M</sup> and other client proteins, and importantly, these effects persisted in tumors for 3-6 days after a single drug dose. 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 relative to normal tissues, with a tumor half-life of 58 hr versus 3-6 hr in liver, lung and plasma, and the tumor concentration remained 215-fold higher than the average *in vitro* IC<sub>50</sub> (72 hr) for a panel of NSCLC cell lines, even 6 days after a single drug dose.

**Conclusions:** Taken together, these results demonstrate that STA-9090 is a highly potent Hsp90 inhibitor that selectively accumulates in tumors and induces long-lasting client protein degradation, cell cycle arrest, increased apoptosis and tumor growth inhibition in a lung cancer xenograft model. Our results suggest that an infrequent dosing schedule may have clinical activity in cancer patients.

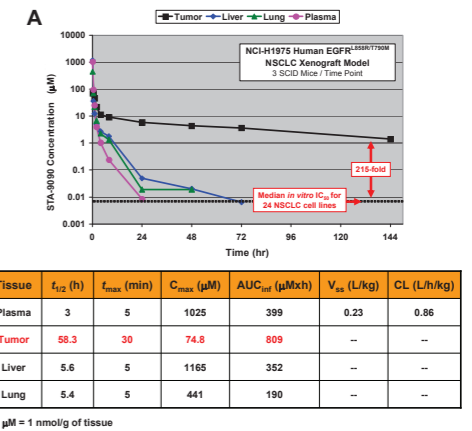
## Results



**Figure 2. STA-9090 displays 5-fold superior *in vitro* cytotoxicity relative to 17-AAG and induces Hsp90 client protein degradation in a NSCLC cell line**  
The erlotinib-resistant NCI-H1975 human NSCLC cell line was selected to study the *in vitro* and *in vivo* activity of STA-9090. (A) NCI-H1975 cells were treated with STA-9090 or 17-AAG for 72 hr and cell viability was measured by Alamar blue assay. IC<sub>50</sub> values were determined using XLfit. STA-9090 was found to be 5-fold more potent than 17-AAG. The median *in vitro* cytotoxicity IC<sub>50</sub> values for STA-9090 and 17-AAG against a panel of 24 human NSCLC cell lines were 6.5 nM and 30.5 nM, respectively (data not shown). (B) NCI-H1975 cells were treated with varying concentrations of STA-9090 for 18 hr and expression of Hsp90 client proteins was measured by Western blotting.

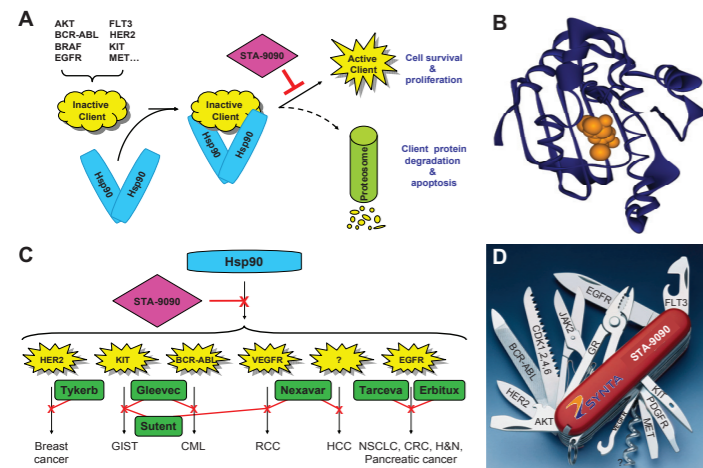


**Figure 5. STA-9090 treatment results in long-lasting downregulation of Hsp90 client proteins in tumors**  
(A) In the NCI-H1975 xenograft model, STA-9090 and 17-AAG were *i.v.* dosed a single time at their HNSTDs and tumors (N = 4/group) were removed at 24, 72 and 144 hr after treatment and analyzed for Hsp90 client protein expression by Western blotting. (B) Western blots were quantified using ImageJ and expression levels are presented as a percentage of expression in untreated tumors. Different Hsp90 client proteins display distinct degradation and resynthesis kinetics. STA-9090 consistently had a greater impact on client protein expression than 17-AAG. Importantly, some changes in client protein expression were still evident and even maximal at 3-6 days after a single drug treatment. Error bars represent +/- SD.

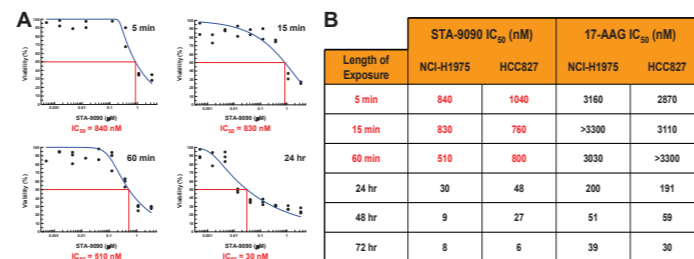


**Figure 7. STA-9090 accumulates in tumors relative to normal tissues**  
(A) STA-9090 was *i.v.* dosed a single time at its HNSTD and its pharmacokinetics were determined in tumor, liver, lung and plasma over a 6 day time period after treatment (N = 3/timepoint). At 144 hr after dosing, the tumor concentration of STA-9090 remained 215-fold higher than the median *in vitro* cytotoxicity IC<sub>50</sub> of 6.5 nM for STA-9090 against a panel of 24 human NSCLC cell lines (data not shown). Error bars not shown for clarity. (B) Summary of the pharmacokinetic parameters of STA-9090 in the NCI-H1975 xenograft model. The half-life of STA-9090 was 10-19 times longer in tumors relative to normal tissues and plasma.

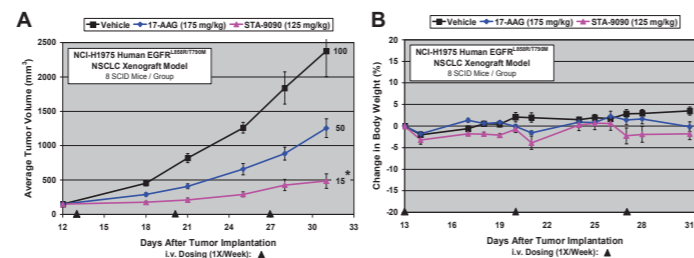
## Introduction



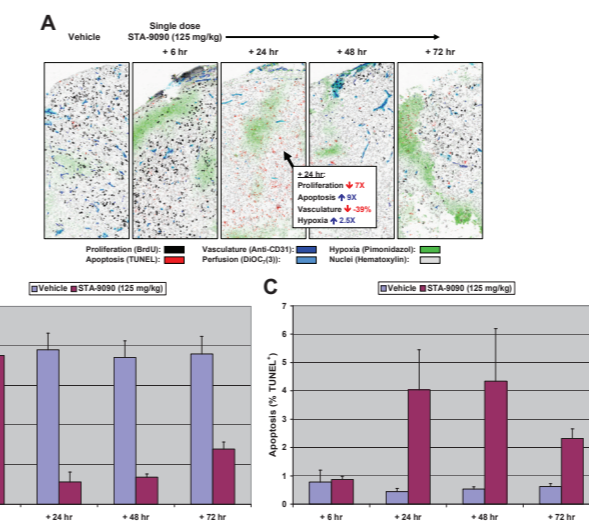
**Figure 1. STA-9090: A Swiss Army knife for the treatment of cancer**  
(A) The molecular chaperone Hsp90 plays a key role in regulating the correct folding, stability and activity of many important signal transduction molecules that have been implicated in the pathophysiology of cancer. For this reason, 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 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 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.



**Figure 3. STA-9090 *in vitro* cytotoxicity requires only 5-60 min drug exposure**  
(A) NCI-H1975 cells were treated with STA-9090 for varying amounts of time, washed to remove the drug, and cell viability was measured at 72 hr by Alamar blue assay. IC<sub>50</sub> values were determined using XLfit. Treatment with STA-9090 for periods as short as 5 min still resulted in an IC<sub>50</sub> < 1000 nM, which is below the drug concentration present *in vivo* in xenograft tumors several days after a single drug dose (see Figure 7). (B) The *in vitro* cytotoxicity IC<sub>50</sub> values for STA-9090 and 17-AAG on the NCI-H1975 and erlotinib-sensitive HCC827 (EGFR<sup>WT</sup>) cell lines were determined as above.



**Figure 4. STA-9090 displays greater *in vivo* efficacy than 17-AAG without increased toxicity**  
The *in vivo* efficacy of STA-9090 was examined in 29 different mouse syngeneic and xenograft models of solid tumor and hematological malignancies. These models varied in their sensitivity to STA-9090, with different models achieving complete responses, partial responses, stable disease or progressive disease (data not shown). The erlotinib-resistant NCI-H1975 NSCLC xenograft model in C.B-17 SCID mice was found to be moderately sensitive to STA-9090 (stable disease) and was therefore selected for further pharmacokinetic-pharmacodynamic studies. (A) In this model, STA-9090 and 17-AAG were intravenously (*i.v.*) dosed 1X/week (arrowheads) at their empirically determined highest non-severely toxic doses (HNSTDs) on a 1X/week schedule for 3 weeks. Compounds were formulated in 10/18 DRD (10% DMSO, 18% Cremophor RH40, 3.6% dextrose in water). STA-9090 displayed significantly greater efficacy than 17-AAG, with %T/C values of 15 and 50, respectively (P < 0.05; one-way ANOVA). (B) Both drugs were well tolerated, with only minimal effects on cumulative average body weight changes over the course of the study. Error bars represent +/- SEM.



**Figure 6. STA-9090 treatment results in long-lasting inhibition of proliferation and induction of apoptosis in tumors**  
(A) In the NCI-H1975 xenograft model, STA-9090 was *i.v.* dosed a single time at its HNSTD and tumors (N = 8/group) were removed at 6, 24, 48 and 72 hr after treatment. Immunohistochemistry was performed on cryosections for markers of proliferation (bromodeoxyuridine), apoptosis (TUNEL), vasculature (CD31), hypoxia (pimonidazole) and perfusion (DiOC7(3)), and results were quantitated using a robotic fluorescent microscope and customized ImageJ software by Cabenda Pharmaceuticals Research (Vancouver, BC, Canada). Maximal effects were observed at the 24 hr time point, reflecting decreased proliferation and microvascular density and increased apoptosis and hypoxia within the viable regions of tumors. (B) Proliferation in STA-9090 and vehicle-treated tumors was quantitated by measuring the percentage of bromodeoxyuridine-positive cells within viable tumor areas. (C) Apoptosis in STA-9090 and vehicle-treated tumors was quantitated by measuring the percentage of TUNEL-positive cells within viable tumor areas. Error bars represent +/- SED.

## 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.
- *In vitro* in an erlotinib-resistant NSCLC cell line, STA-9090 demonstrated 5-fold greater potency than 17-AAG and rapidly induced the degradation of critical Hsp90 client proteins. As little as 5 min STA-9090 exposure gave an *in vitro* cytotoxicity IC<sub>50</sub> < 1 µM, suggesting very brief exposure may be sufficient to affect tumor growth.
- *In vivo* in an erlotinib-resistant NSCLC xenograft model, 1X/week dosing with STA-9090 demonstrated superior efficacy to 17-AAG and rapidly induced the degradation of critical Hsp90 client proteins in tumors. Importantly, decreased client protein expression, decrease proliferation and increased apoptosis in tumors persisted for up to 3-6 days after a single drug dose.
- *In vivo*, STA-9090 accumulated in tumors relative to normal tissues, and the tumor concentration remained above the *in vitro* IC<sub>50</sub> for at least 6 days after a single drug dose.
- Our results demonstrate that STA-9090 is a highly potent and rapidly acting Hsp90 inhibitor that can induce long-lasting changes *in vivo* in Hsp90 client protein expression, proliferation and apoptosis in tumors. These results are consistent with an infrequent dosing schedule in the clinic.
- STA-9090 is currently being examined in multiple Phase 1/2 clinical trials in subjects with solid tumor and hematological malignancies.

## References

- 1) Lin, T.-Y., Bear M., Du Z., Foley K.P., Ying W., Barsoum J., and London C. 2008. The novel HSP90 inhibitor STA-9090 exhibits activity against Kit-dependent and -independent malignant mast cell tumors. *Exp. Hematol.* 36:1266-77.
- 2) McCleese J.K., Bear M.D., Fossey S.L., Mihalek R.M., Foley K.P., Ying W., Barsoum J., and London C.A. 2009. The novel HSP90 inhibitor STA-1474 exhibits biologic activity against osteosarcoma cell lines. *Int. J. Oncol.* 125:2792-801.

