Preclinical Evaluation of the Potent 2nd Generation Small-Molecule Hsp90 Inhibitor STA-9090 in Hematological Cell Lines

Weiwen Ying, David Proia, Suqin He, Jim Sang, Kevin Foley, Zhenjian Du, Ronald K Blackman, Jessica Kirshner, Yumiko Wada, Lijun Sun and Keizo Koya

Synta Pharmaceuticals, 45 Hartwell Ave., Lexington, MA 02421 USA

Abstract # 2899

STA-9090 is a potent, second generation, small-molecule Hsp90 inhibitor, with a chemical structure unrelated to the first-generation, ansamycin family of Hsp90 inhibitors. In preclinical *in vitro* and *in vivo* studies, STA-9090 has shown potency up to 100 times greater than the first-generation Hsp90 inhibitors against a wide range of solid and hematological cancer types including those resistant to imatinib, sunitinib, erlotinib, and dasatinib. STA-9090 is currently being evaluated two Phase 1 and four Phase 2 trials (non-small cell lung, GIST, colon, and gastric) in solid tumor cancers; and two trials in hematologic cancers. Additional Phase 2 trials in several other indications are planned for 2H 2010.

Inhibition of Hsp90 by STA-9090 results in the destabilization of a broad range of oncogenic kinases often overexpressed or mutated in hematological cancers. For example, the nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) expressed in the anaplastic large cell lymphoma (ALCL) cell line Karpas 299, is degraded rapidly in the presence of STA-9090 *in vitro*, resulting in the loss of viability. Similar results were shown in other NPM-ALK driven ALCL cells including SU-DHL-1 and SR-786 with IC_{50} less than 20 nM. Stability of other kinases common to hematological malignancies, such as Bcr-Abl, FLT3 and c-Kit, were also shown to be highly sensitive to STA-9090, resulting in potent cell death of cell lines addicted to signaling by these kinases.

In vivo, STA-9090 was highly effective in a subcutaneous xenograft model of diffuse large B-cell lymphoma SU-DHL-4 with resulting %T/C values of 26, 4, -90 and -93 when dosed at 25, 50, 75 and 100mg twice per week, respectively. Importantly, 75 and 100mg/kg STA-9090 dosed 2 times per week for a total of 3 weeks (150 and 200mg/kg weekly) resulted in 25% and 50% of the animals in each group being free of tumors by the end of the study, respectively. MV4-11, an AML ($FLT3^{ITD}$) cell line, turned out to be one of the most sensitive xenograft models to STA-9090 treatment. STA-9090 at 100mg/kg or125mg/kg once weekly was highly efficacious with 37.5% of mice achieving tumor free with acceptable toxicity at the end of the 3-week treatment period.

In conclusion, STA-9090 exhibits preferable biological profiles both *in vitro* and *in vivo* in treating hematological malignances. Clinical studies for using STA-9090 both once weekly and twice weekly are ongoing.

Introduction

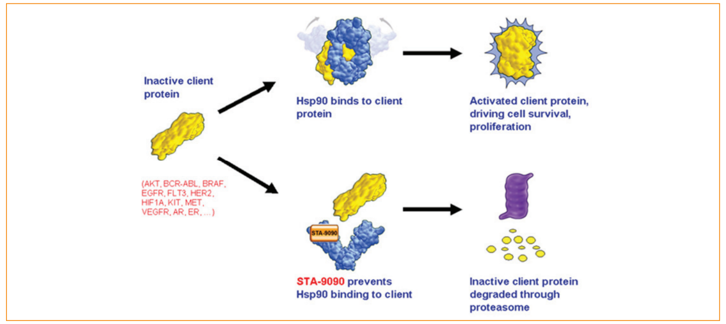
STA-9090 is a potent, second-generation, small-molecule heat shock protein 90 (Hsp90) inhibitor, being developed for treating multiple solid tumor and hematologic cancers.

STA-9090 was discovered and developed internally at Synta and has a chemical structure unrelated to the first-generation, ansamycin family of Hsp90 inhibitors such as 17-AAG or IPI-504. In preclinical studies, STA-9090 has shown potency up to 100 times greater than the first-generation Hsp90 inhibitors as well as activity against a wider range of kinases. In *in vitro* and *in vivo* models, STA-9090 has shown potent activity against a wide range of cancer types, including lung, prostate, colon, breast, gastric, pancreatic, gastrointestinal stromal tumors (GIST), melanoma, acute myeloid leukemia, chronic myeloid leukemia, Burkitt's lymphoma, diffuse large B-cell lymphoma, and multiple myeloma - as well as potent activity against cancers resistant to imatinib (Gleevec®), sunitinib (Sutent®), erlotinib (Tarceva®), and dasatinib (Sprycel®).

Mechanism of Action

STA-9090 potently inhibits Hsp90, a chaperone protein required for the proper folding and activation of other cellular proteins, particularly kinases. Many of these "client proteins", such as AKT, BCR-ABL, BRAF, KIT, MET, EGFR, FLT3, HER2, PDGFRA, VEGFR, have been shown to be critical to cancer cell growth, proliferation, and survival. In preclinical studies, inhibiting Hsp90 causes the degradation of multiple client proteins and leads to cancer cell death. Because mutated kinases which no longer respond to treatment with kinase inhibitors remain dependent on Hsp90 for their activity, inhibiting Hsp90 offers the potential for treating cancers that have become resistant to targeted therapies such as kinase inhibitors. We believe that inhibiting kinases indirectly, by disrupting the chaperone activities of Hsp90, provides two advantages: first, a means to simultaneously attack multiple cancer-promoting kinases; and second, an ability to kill tumor cells with mutated kinases that have lost responsiveness to a direct kinase inhibitor.

Figure 1. Mechanism of STA-9090 Action



STA-9090 Possesses Potent *In Vitro* Activity in Hematological Cancer Cells

Table 1. STA-9090 Cytotoxicity in a Panel of Human Hematological Cell Lines

Cell Line	Cancer	IC_{50} (nM)	
		STA-9090	17-AAG
HL60	Myeloid Leukemia	44	287
CCRF-HSB-2	T Cell Leukemia	2	273
CCRF-SB	B Cell Leukemia	7	718
Daudi	B Cell Lymphoma	9	2911
SU-DHL-4	B Cell Lymphoma	20	2919
SU-DHL-8	B Cell Lymphoma	10	317
KU812	CML ($Bcr\text{-}Abl^+$)	6	318
K562	CML ($Bcr\text{-}Abl^+$)	20	265
KCL-22	CML ($Bcr\text{-}Abl^+$)	13	749
LAMA-84	CML ($Bcr\text{-}Abl^+$)	10	287
CML-T1	CML ($Bcr\text{-}Abl^+$)	9	1271
MV4-11	AML ($Flt3^{ITD}$)	<4	35
MOLM-13	AML ($Flt3^{ITD}$)	5	90
RS-4-11	AML ($Flt3^+$ Pro-B)	8	1282
THP-1	AML ($Flt3^+$ monocyte)	26	820
Kasumi-1	AML ($c\text{-}Kit^{N822K}$)	4	483
Kasumi-3	AML ($c\text{-}Kit^+$)	<12	618
CMK	AML ($c\text{-}Kit^+$)	5	516
P815	Mastocytoma ($c\text{-}Kit^{D814V}$)	16	2215
Karpas-299	ALCL ($NPM\text{-}ALK^+$)	10	452
SU-DHL-1	ALCL ($NPM\text{-}ALK^+$)	14	299
SR-786	ALCL ($NPM\text{-}ALK^+$)	16	506
RPMI-8226	Myeloma	<36	73

STA-9090 is highly potent in hematological cancer cell lines with a median IC_{50} of 10 nM.

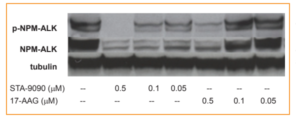
Figure 2. Apoptosis Induction by STA-9090 in Human HSB2 T-Cell Leukemia Cells

(A) STA-9090 vs. 17-AAG in inducing apoptosis in HSB2 cells

(B) STA-9090 vs. 17-AAG in activation of caspase-3 in HSB2 cells

STA-9090 potently induces apoptosis and activates caspase-3 through internal pathway.

Figure 3. STA-9090 Induced NPM-ALK Degradation in Karpas 299 Cells



Karpas 299 is a T-anaplastic large cell lymphoma cell line. The majority of anaplastic large cell lymphomas (ALCLs) express the nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) fusion protein, which is oncogenic due to its constitutive tyrosine kinase activity. Transformation by NPM-ALK not only increases proliferation, but also modifies cell shape and motility in both lymphoid and fibroblastic cells. STA-9090 is potent in inducing NPM-ALK degradation (Figure 3) and cell growth inhibition (see Table 1).

STA-9090 is Efficacious in NPM-ALK Driven Tumor Xenografts

Hsp90 is over-expressed in primary and cultured ALK-positive and ALK-negative ALCL cells. Here we investigated the action of STA-9090 on NPM-ALK expression in the ALCL line SU-DHL-1, established from the pleural effusion of a patient known to carry the t(2;5)(p23;q35) NPM-ALK fusion gene.

STA-9090 is highly efficacious in SU-DHL-1 xenografts. Tumor regression (T/C=-16.9%) was observed after one single dose of STA-9090 at 150mg/kg. Measurements were taken on Day 0 & Day 1.

Figure 4. Treatment of STA-9090 in SU-DHL-1 Xenografts

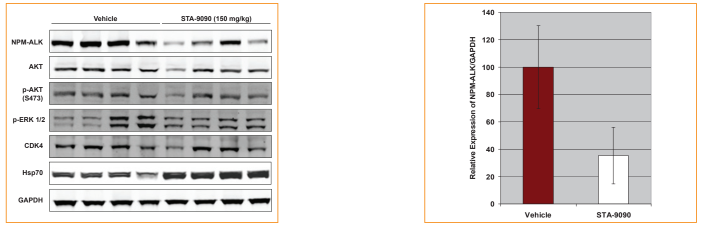


Figure 4. (A) STA-9090 was dosed at 150mg/kg and tumor samples were collected 24 hours later. Western blot analysis showed effective destabilization of the NPM-ALK fusion protein as well as inactivation of its effector, p-AKT; (B) Quantitation of NPM-ALK expression from vehicle or STA-9090 treated tumors, normalized to the housekeeping protein GAPDH.

Dose Dependent Tumor Growth Suppression by STA-9090 in a B-Cell Lymphoma Model

SU-DHL-4 is a diffuse large B-cell lymphoma cell line and known to over-express anti-apoptotic protein BCL-2. Here we investigated the anti-cancer effect of STA-9090 in this DLBCL xenograft model.

STA-9090 is highly efficacious in SU-DHL-4 xenografts. Complete responses were observed in the twice weekly dosing regimen.

Figure 5. Dose and Schedule Dependence Studies of STA-9090 in SU-DHL-4 Xenografts

Figure 5. A diffuse large B-cell lymphoma model was established *in vivo* via the SU-DHL-4 lymphoma xenograft, and displayed dose dependent growth suppression in response to STA-9090. (A) STA-9090 was dosed twice weekly at concentration indicated. STA-9090 at 25, 50, 75 and 100 mg/kg was highly efficacious in this model, with T/C (%) values of 26, 4, -90 and -93, respectively. Importantly, the two highest dose groups resulted in 25% (75mg/kg) and 50% (100mg/kg) of the animals in each group being free of tumors by the end of the study, respectively; (B) Similar studies were performed in SU-DHL-4 xenografts, with dosing at once per week. One out of fifteen mice in the 200mg/kg treatment group showed more than 20% body weight loss. STA-9090 showed dose dependence in once weekly dosing regimen, although the responses were not as remarkable as the twice weekly dosing regimen.

STA-9090 Blocks Tumor Progression in the MV4-11 AML Xenograft Model

In 15% to 30% of patients with acute myeloid leukemia (AML), aberrant proliferation is a consequence of a juxtamembrane mutation in the FLT3 gene, causing constitutive kinase activity. MV4-11 is an AML cell line expressing FLT3 internal tandem duplications (ITD).

Figure 6. Complete Response to STA-9090 Was Achieved in MV4-11 Xenografts

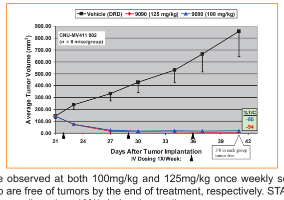


Figure 6. Significant tumor regressions were observed at both 100mg/kg and 125mg/kg once weekly schedule in the $FLT3^{ITD}$ MV4-11 AML xenograft model. Three out of eight mice in either group are free of tumors by the end of treatment, respectively. STA-9090 was well tolerated in the MV4-11 model with only minimal effects on body weight changes (less than 10%) during the studies.

Figure 7. Impact of Dosing Frequency

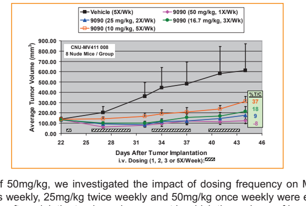


Figure 7. With the same weekly amount of 50mg/kg, we investigated the impact of dosing frequency on MV4-11 xenografts. Doses of STA-9090 at 10mg/kg 5 times weekly, 16.7mg/kg 3 times weekly, 25mg/kg twice weekly and 50mg/kg once weekly were efficacious with T/C (%) values of 37, 18, 9 and -8, respectively. For MV4-11 AML xenograft model, the tendency is apparent in which the regimen of less dosing frequency at higher dosage results in better response than that of higher dosing frequency at lower dosage.

Biomarker Studies in MV4-11 AML Xenografts

Using the highly sensitive MV4-11 xenograft model, we investigated the biomarkers responding to the treatment of STA-9090 in mice.

Tumor regression (T/C=-30%) in mice was observed 48 hours after treatment (35mg/kg once).

Figure 8. Possible Biomarkers in PBMC

PBMC samples from tumor bearing mice were analyzed for Hsp70 and known Hsp90 client proteins.

(A) Hsp70 in PBMC is induced by a single dose of STA-9090

(B) Client proteins LCK and RSK1 in PBMC are not degraded by a single dose of STA-9090

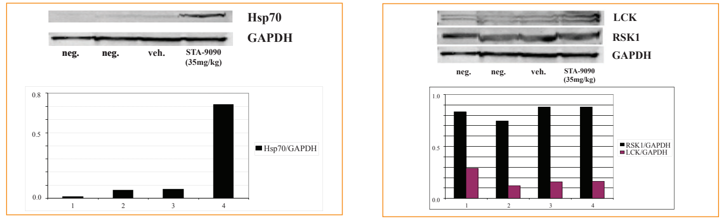


Figure 8. (A) It is well-documented that Hsp70 is induced by Hsp90 inhibition through HSF-1 up-regulation. More than 10-fold increase of Hsp70 level was observed with STA-9090 treatment; (B) LCK and RSK1 are known Hsp90 clients. No detectable degradation of these two client proteins was observed in PBMC samples.

Figure 9. Possible Biomarkers in MV4-11 AML Tumor Tissues

Tumor tissues were analyzed for Hsp70 and known Hsp90 client proteins.

(A) Hsp70 and LCK in tumor are regulated by STA-9090 in MV4-11 xenografts

(B) Client proteins RSK1 and CDK4 in tumor are degraded by a single dose of STA-9090 in MV4-11 xenografts

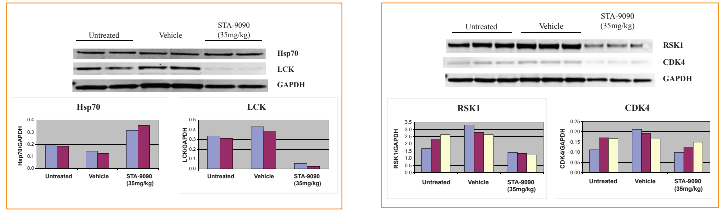


Figure 9. (A) Hsp70 in tumor tissue was clearly up-regulated and is about 2-3 fold higher when compared to untreated and vehicle groups. Meanwhile LCK was degraded significantly with STA-9090 treatment; (B) both client proteins RSK1 and CDK4 were clearly degraded although the effect was less dramatic compared to LCK.

Conclusions

- STA-9090 is more potent than 17-AAG in killing and inducing apoptosis in hematological cancer cell lines.
- STA-9090 causes death of hematological cancer cells such as HSB2 which lack known oncogenic Hsp90 client proteins by directly inducing apoptosis through an intrinsic pathway.
- NPM-ALK fusion protein is major driver in ALCL. STA-9090 is highly potent in the SU-DHL-1 xenograft model.
- Treatment of STA-9090 induced complete responses in a B-cell lymphoma model (SU-DHL-4 DLBCL) and an AML model (MV4-11).
- Dosing frequency has great impact on the tumor growth response.
- STA-9090 inhibition induced client protein degradation and Hsp70 up-regulation in tumor tissue. Hsp70 up-regulation but not client protein degradation was observed in normal tissue (PBMC). Both effects are being evaluated as potential biomarkers in ongoing clinical trials.

SYNTA PHARMACEUTICALS®

ASH 2010, Orlando, FL, December 4-7, 2010

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