The novel Hsp90 inhibitor STA-9090 has potent anticancer activity in *in vitro* and *in vivo* models of lung cancer

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RATIONALE

- EGFR kinase domain activating mutations are found in ~10% of NSCLC patients in the U.S. (1)
- EGFR tyrosine kinase inhibitors (TKIs) are the primary choice for the subset of patients with EGFR mutations, but resistance to TKIs emerges over time (2)
- Higher levels of active Hsp90 are found in cancer cells relative to normal cells (3)
- Many oncogenic kinases depend on Hsp90 for conformational maturation and stability (4,5)
- EGFR mutants are more dependent on Hsp90 for stability than wild type EGFR (6)
- Reagents to overcome resistance to EGFR inhibitors are under intense investigations
- Hsp90 inhibitors overcome resistance to EGFR inhibitors by both T790M secondary mutation and by kinase switch (7)
- Tumor cells are known to develop resistance to ansamycin class Hsp90 inhibitors (8,9,10)
- The limited clinical efficacy, as well as formulation and toxicity issues, of ansamycin class Hsp90 inhibitors such as 17-AAG has prompted the search for more potent inhibitors with improved pharmaceutical properties

RESULTS

STA-9090 is more potent *in vitro* than 17-AAG in NSCLC

Table 1. Comparison of 17-AAG and STA-9090 *in vitro* cytotoxicity in NSCLC cell lines

Cell Line	EGFR	ERBB2	KRAS	Others	Erlotinib IC_{50}	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_E746_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 I T790M	Wild-type	Wild-type		Resistant	34	3
DFCI-L0511	Del L747_E746_A750P	Wild-type	Wild-type		Resistant	111	2
NCI-H1650	Del E746_A750	Wild-type	Wild-type		Resistant	7	7
NCI-H1781	Wild-type	G778insV_GC	Wild-type		Resistant	22	2
NCI-H1734	Wild-type	Wild-type	G12C		Resistant	96	12
A549	Wild-type	Wild-type	G12S		Resistant	75	22
NCI-H460	Wild-type	Wild-type	Q61H		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	Wild-type	NRAS (Q61K)	Resistant	36	6
NCI-H596	Wild-type	Wild-type	Wild-type	BRAF (G469V)	Resistant	27	6
NCI-H1819	Wild-type	Wild-type (Amp)	Wild-type	PIK3CA	Resistant	749	7
NCI-H1703	Wild-type	Wild-type	Wild-type	PDGFRA (Amp)	Resistant	3	3
NCI-H596	Wild-type	Wild-type	Wild-type	RB Null	Resistant	3,500	7
NCI-H522	Wild-type	Wild-type	Wild-type		Resistant	7	6
NCI-H838	Wild-type	Wild-type	Wild-type		Resistant	4	>1
Calu-3	Wild-type (Amp)	Wild-type (Amp)	Wild-type		Resistant**	16	9

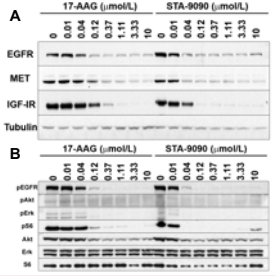
* Units: nmol/L. **Results vary between reports

STA-9090 potently inhibits the growth of Ba/F3 cells ectopically expressing EGFR mutants found in patients with NSCLC

EGFR Activating Mutations					EGFR Activating Mutations in *cis* with T790M				
EGFR Mutation	Erlotinib IC_{50}	CL-387,785 IC_{50}	17-AAG IC_{50}	STA-9090 IC_{50}	EGFR Mutation	Erlotinib IC_{50}	CL-387,785 IC_{50}	17-AAG IC_{50}	STA-9090 IC_{50}
Del E746_A750	4	1	92	*4*	Del E746_A750/T790M	>10000	294	73	*10*
Del S752_I759	37	<1	180	*12*	Del S752_I759/T790M	>10000	537	88	*7*
Del L747_A750insP	5	21	67	*4*	Del L747_A750insP/T790M	>10000	445	38	*4*
Del L747_A753insS	1	<1	136	*3*	Del L747_A753insS/T790M	>10000	258	119	*4*
Del E746_S752insV	25	274	58	*5*	Del E746_S752insV/T790M	>10000	756	40	*2*
L858R	16	5	246	*7*	L858R/T790M	>10000	950	155	*12*
A767_V769dupASV	>3000	427	2262	*34*					
H773_V774insH	>3000	229	110	*40*					
D770_N771insNPG	>3000	7	12	*1*					

*Units: nmol/L

STA-9090 depletes client proteins and suppresses signaling cascade better than 17-AAG in NSCLC with EGFR mutation



A: H1975 receptors

EGFR - L858R/T790M

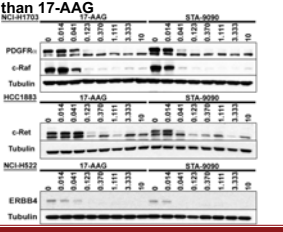
MET - wildtype

IGF-1R - wildtype

B: H1975 signaling

Treated for 24hrs with doses indicated

STA-9090 depletes PDGFR, c-Ret, and ERBB4 better than 17-AAG



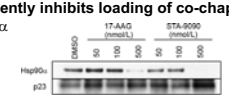
Treated for 24hrs with doses indicated

PDGFRα - wildtype

c-Ret- wildtype

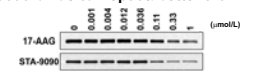
ERBB4 - wildtype

STA-9090 potently inhibits loading of co-chaperone p23 to Hsp90α



Lysates were made from NCI-H1975 cells treated with indicated concentrations of Hsp90 inhibitors for 2 hours. Lysates were subject to immunoprecipitation with anti-p23 antibody and Western blot was performed with indicated antibodies.

STA-9090 binds to Hsp90α better than 17-AAG



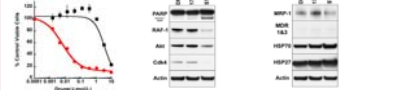
To investigate if STA-9090 had a higher binding affinity to Hsp90 than 17-AAG, a competitive binding assay was performed using biotinylated geldanamycin (biotin-GM) conjugated to streptavidin coated beads. Incubation of Hsp90 isolated from NCI-H1975 cells with indicated Hsp90 inhibitor concentrations inhibited the binding of Hsp90 to biotin-GM.

STA-9090 is efficacious in lung cancer cells resistant to 17-AAG

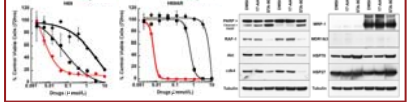
[NSCLC]

MTS assays: 72hrs

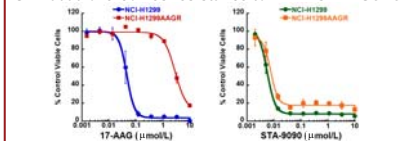
Western: Treated for 24hrs 500nmol/L STA-9090 or 17-AAG



[SCLC]

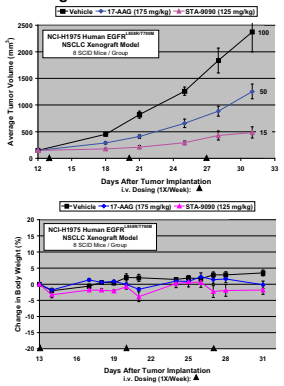


STA-9090 overcomes resistance to 17-AAG in NSCLC



NCI-H1299 was grown in increasing concentrations of 17-AAG for 2 months to obtain 17-AAG resistant cell line NCI-H1299AAGR

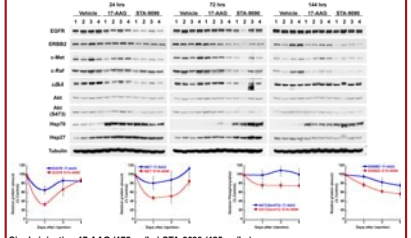
STA-9090 is more efficacious *in vivo* than 17-AAG when dosing 1X/week at HNSTD*s



*Highest non-severely toxic dose

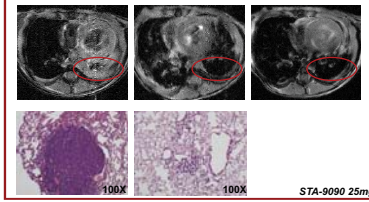
Both STA-9090 and 17-AAG were well tolerated when dosing 1X/week at HNSTDs in C.B-17 SCID mice subcutaneously injected with NCI-H1975 cells

STA-9090 suppresses client proteins better than 17-AAG in NCI-H1975 xenograft model



Single injection 17-AAG (175mg/kg) STA-9090 (125mg/kg)

Inducible bitransgenic mouse expressing $EGFR^{delE746\text{-}S752/T790M}$ responds to STA-9090 treatment



CONCLUSIONS

- STA-9090 demonstrated potent cell killing across a panel of NSCLC cell lines (median IC50 = 6.5nmol/L) compared to 17-AAG (median IC50 = 30.5nmol/L)
- STA-9090 destabilized EGFR, IGF-1R, c-Met, ERBB4, PDGFRα, and c-Ret at a 3-9 fold lower concentrations than 17-AAG
- Biochemical assays demonstrated that STA-9090 competes with geldanamycin for binding to N-terminal ATPase pocket of Hsp90
- STA-9090 retained activity against NSCLC cell lines resistant to 17-AAG. Cells selected for 17-AAG resistance were still sensitive to STA-9090
- STA-9090 demonstrated efficacy in both NSCLC and SCLC cell lines expressing MRP1 multiple drug resistant pump that is linked with resistance to adriamycin and CDDP
- STA-9090 was more efficacious than 17-AAG in the NCI-$H1975^{EGFR\text{-}L858R/T790M}$ NSCLC xenograft model, with %T/C values of 15 and 50, respectively (compared at HNSTDs)
- STA-9090 was efficacious in a bitransgenic mouse *de novo* tumor model with inducible expression of $EGFR^{delE746\text{-}S752/T790M}$ in type II lung pneumocytes
- STA-9090 is currently being examined in multiple clinical trials in subjects with solid and hematological tumors

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