HSP90 inhibitor STA-9090 potently suppresses secondary KIT kinase-domain mutations responsible for gastrointestinal stromal tumor (GIST) progression during imatinib therapy

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Background: Most GISTs express mutant KIT or PDGFRA oncoproteins, which are targets of tyrosine kinase inhibitors (TKIs), such as front-line imatinib (IM) or second-line sunitinib (SU). GIST clinical resistance to IM or SU is commonly associated with the acquisition of heterogeneous secondary mutations in the KIT/PDGFRA ATP-binding pocket (ABP) or activation loop (AL), which maintain the constitutively activated state of these kinases. We therefore asked whether the heterogeneous IM-resistant KIT oncoproteins in GIST are uniformly HSP90 clients, and whether they can be inhibited by STA-9090, a synthetic small molecule HSP90 inhibitor that is structurally unrelated to the first-generation natural product-derived ansamycin HSP90 inhibitor 17-AAG.

Results: As many as 8 different secondary KIT IM-resistance mutations (ABP and AL) were detected in individual patients whose GISTs progressed after IM therapy. All mutations were sensitive to STA-9090. STA-9090 was 5-15 fold more potent than 17-AAG against these IM-resistance secondary mutations, and was at least as effective against the primary + secondary (IM-resistant) mutations, in combination, as compared to the primary IM-sensitive mutation alone. STA-9090 also potently inhibited the 17-AAG resistant GIST882B cell line. STA-9090 inhibited growth of GIST882 xenografts. STA-9090 inhibition of GIST growth/survival pathways was restricted to KIT-dependent GISTs.

Conclusions:

1. STA-9090 is uniformly potent (5 -15 fold more so than 17-AAG) against diverse secondary KIT mutations in TKI-resistant GISTs.
2. STA-9090 is effective against TKI sensitive and resistant KIT-dependent GISTs.
3. STA-9090 has *in vivo* activity against GIST although rebound of KIT expression/activation is seen at 24 hours.
4. These data suggest that STA-9090 may have clinical activity against imatinib-resistant GIST.

Table 1: Cell viability IC50s in Ba/F3 cells transformed by KIT mutants with primary mutation only (Ex 9) vs primary + secondary mutations (Ex 9 + V654A).

Model	IC_{50} STA-9090 (nM)	IC_{50} 17-AAG (nM)	IC_{50} IM (nM)
Ba/F3: KIT Exon 9	**15**	200	200
Ba/F3: KIT Exon 9 + V654A (ABP)	**15**	200	>1000

Fig. 1: Novel IM/SU-resistance mutations are inhibited by STA-9090. HEK293 cells were transfected with various KIT mutants identified in clinically-progressing GISTs. 24 hours after transfection, cells were exposed to varying concentrations of STA-9090 for 6 hours. Whole-cell lysates were immunostained for p-KIT(Y703), p-KIT(Y721), total KIT and ß-Actin.
Sunitinib-resistant KIT mutations retain biochemical sensitivity to STA-9090.

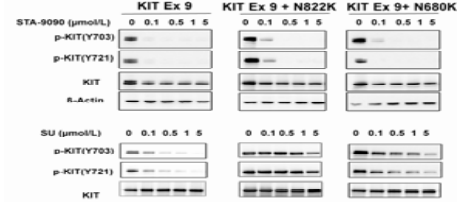


Table 2: Cell viability IC50s in KIT-dependent and KIT-independent (GIST62) GISTs

Cell line	IC50 STA-9090 (nM)	IC50 17-AAG (nM)	IC50 IM (nM)
GIST882: KIT Ex 13	**40**	200	300
GIST882B (17-AAG resistant)	**35**	>1000	300
GIST430: KIT Ex 11 + V654A (ABP)	**20**	300	>1000
GIST48: KIT Ex 11 + D820A (AL)	**20**	100	>1000
GIST62 (KIT-negative)	**>1000**	>1000	>1000

Fig. 2: STA-9090 and IM inhibition of KIT and signaling intermediates (AKT, MAPK) in KIT-dependent GIST882 vs. KIT-independent GIST62.

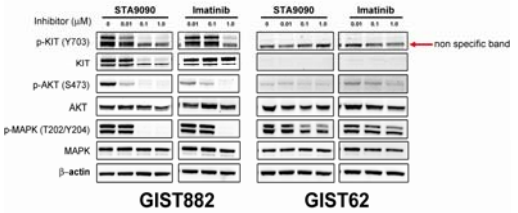


Fig. 3: Cell viability and apoptosis response to STA-9090 and 17-AAG in KIT-dependent GISTs, irrespective of whether IM-sensitive (GIST882) or IM-resistant (GIST48 and GIST430).

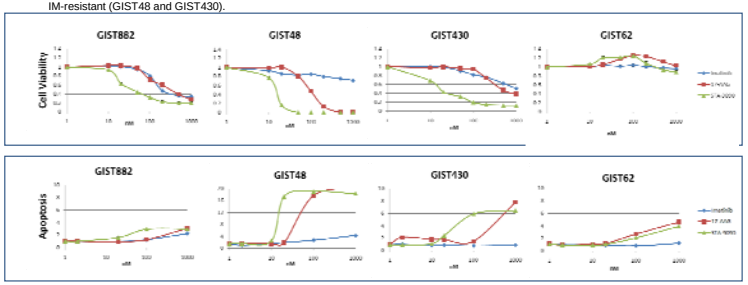
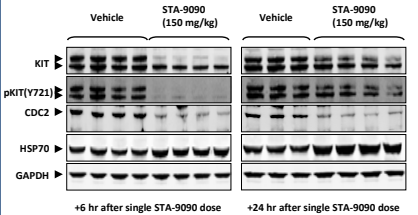
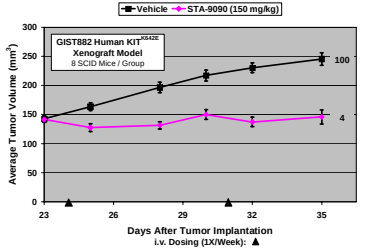
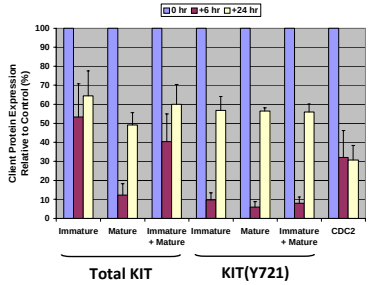
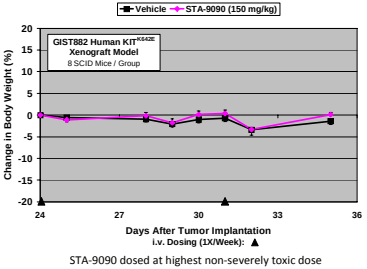


Fig. 4: GIST882 xenografts treated with STA-9090 have biochemical response (A, B) and growth arrest (C), with no associated change in overall mouse body weight (D).









STA-9090 dosed at highest non-severely toxic dose

Methods: KIT and PDGFRA were genotyped in up to 15 metastases from each of 10 patients whose metastatic GIST had progressed after IM therapy. IM-resistant KIT mutations were biochemically profiled for IM and STA-9090 sensitivity using: 1. Ba/F3 and HEK293 cells expressing mutant KIT constructs; 2. GIST cell lines that are KIT-dependent (GIST882) vs KIT-independent (GIST62); and 3. a novel assay of KIT activation after drug treatment in GIST48B (KIT-negative) cells transfected with mutant KIT constructs. STA-9090 effects on proliferation, apoptosis and cell cycle were evaluated in five GIST cell lines, including a KIT-dependent GIST subline (GIST882B) that is resistant to 17-AAG. Further STA-9090 effects on KIT activation and GIST growth *in vivo* were evaluated using a GIST882 xenograft model conducted in C.B-17 SCID mice.