

Abstract # B184

## Hsp90 inhibitor STA-9090 potently suppresses heterogeneous KIT kinase-domain mutations responsible for gastrointestinal stromal tumor 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/PDGFR 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.

**Methods:** KIT and PDGFRA were genotyped in up to 15 metastases from each of 10 patients whose metastatic GIST had progressed after IM therapy. Kinase mutants were biochemically profiled for IM and STA-9090 sensitivity in: 1. Ba/F3 cells transformed by mutant KIT constructs; 2. GIST cell lines (IM-sensitive and IM-resistant); and 3. a novel assay measuring inhibition of kinase phosphorylation after drug treatment in GIST48B (KIT-negative) GIST cells transfected with mutant KIT constructs. Drug 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.

**Results:** As many as 8 different secondary KIT IM-resistance mutations (both 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-resistant KIT 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 (Table). STA-9090 also potently inhibited the 17-AAG resistant GIST882B cell line.

**Conclusions:** STA-9090 was more potent than 17-AAG against a panel of KIT mutations found in TKI-resistant GISTs. STA-9090 potency was undiminished against combination activating KIT mutations (including secondary kinase-domain resistance

mutations), as typically occur in IMresistant GIST clones. Based on these results, we hypothesize that STA-9090 might have broad clinical activity against IM-resistant GIST.

**Table: IC50 (nM) for GIST viability: HSP90-inhibition vs. imatinib**

<b>GIST Line</b>	<b>STA-9090</b>	<b>17-AAG</b>	<b>Imatinib</b>
GIST882: KIT Ex 13	40	200	300
GIST882B	35	>1000	300
GIST430: KIT Ex 11 + V654A (ABP)	20	300	>1000