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## **Multimodal action of the Hsp90 inhibitor STA-9090 in treating cancer cells with activated JAK/STAT signaling.**

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Heat Shock Protein 90 (Hsp90) is a molecular chaperone that mediates the post-translational stability of its protein substrates (“client proteins”). Cancer cells are particularly sensitive to Hsp90 inhibition because many client proteins play critical oncogenic roles. Accordingly, the potent synthetic small-molecule Hsp90 inhibitor STA-9090 shows *in vitro* activity in the low nM range against a broad range of tumor cell lines and it currently is in multiple Phase 1 and Phase 2 clinical trials for solid tumor and hematologic malignancies.

The JAK kinases are established Hsp90 client proteins and there is accumulating evidence that constitutive activation of JAK signaling occurs in a wide variety of cancer types. In particular, mutations in JAK2 can result in the constitutive phosphorylation and activation of the transcription factors STAT3 and STAT5, thereby leading to oncogenic growth. Here, we show, both *in vitro* and *in vivo*, that STA-9090 potently inhibits the proliferation of numerous solid tumor and hematological cancer cell lines that are dependent upon persistent JAK/STAT signaling for growth and survival. Western blot analysis demonstrates that STA-9090 blocks endogenous and IL-6-induced JAK/STAT signaling in these cells. Importantly, STA-9090 treatment leads to sustained depletion of JAK2, producing a prolonged loss of STAT phosphorylation/activity and reduced expression of STAT target genes such as the oncogenes PIM1/2 and SOCS1/3. In contrast, treatment with the pan-JAK kinase inhibitor P6 leads only to a transient effect on these processes. RNA profiling studies on HEL92.1.7 cells, which express the constitutively activated JAK2<sup>V617F</sup> mutation, demonstrate that STA-9090 has a more profound activity on these cells than P6. In addition to the JAK/STAT targets that both drugs influence, only STA-9090 treatment leads to the loss of cyclins and cyclin-dependent kinases. Such changes reflect the G<sub>1</sub> and G<sub>2</sub>/M arrest typically induced by STA-9090 in multiple cancer cell types. Additional client proteins are affected as well and STA-9090 treatment leads to apoptosis. *In vivo*, xenograft models of human MV4-11 AML cells (which carry a FLT3-ITD mutation that drives constitutive activation of STAT5) demonstrate that a single dose of STA-9090 represses activation of STAT5 and suppresses expression of the cyclin-dependent kinase CDK1 for more than three days, leading to tumor regression. STAT5 signaling, but not CDK1 expression, returns by the sixth day without a change in tumor volume. This suggests that the coordinate loss of cell growth and cell division signals orchestrated by STA-9090, with only intermittent dosing,

is responsible for reducing and maintaining tumor regression. Thus, inhibition of Hsp90 activity presents a new method for combating diseases dependent on constitutive JAK/STAT signaling, with STA-9090 having advantages over JAK-specific inhibitors.