The interplay between inhibition of JAK2 and HSP90

Jordan S. Fridman and Nicholas J. Sarlis*

Incyte Corporation; Wilmington, DE USA

Commentary to: Weigert O, Lane AA, Bird L, Kopp N, Chapuy B, van Bodegom D, et al. Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition. J Exp Med 2012; 209:259–73; PMID:22271575; http://dx.doi.org/10.1084/jem.20111694

A recent article by Weigert et al. published in *The Journal of Experimental Medicine* described the in vitro generation of synthetic mutations in Janus kinase 2 (JAK 2) that decreased the potency of JAK2 (or JAK1/JAK2) inhibitors in artificial systems. The authors found that heat shock protein 90 (HSP90) inhibitors circumvented the potency shift and suggested that HSP90 inhibition may abrogate JAK inhibitor resistance in these experimental systems. However, the clinical relevance of these laboratory-generated JAK2 mutations, which have not been identified to-date in patients treated with JAK inhibitors, and the therapeutic potential of HSP90 inhibitors in diseases involving aberrant JAK-STAT signaling remain to be determined.

Alterations in Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling have been identified in several disease states, including inflammatory conditions (rheumatoid arthritis, psoriasis) and some cancers [myeloproliferative neoplasms (MPNs), other hematologic cancers, pancreatic cancer]. Thus, JAK inhibitors are under very active investigation as novel therapeutic agents with the potential for broad application. The first such inhibitor to receive US Food and Drug Administration (FDA) approval is ruxolitinib (Incyte Corporation) for the treatment of patients with intermediate or high-risk myelofibrosis (MF).[1,2] This oral, small-molecule, JAK1 and JAK2 inhibitor is also being investigated in other malignancies.[3]

A recent article published in *The Journal of Experimental Medicine*, "Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition," described the in vitro generation of three synthetic mutations in the JAK2 kinase domain—G935R, Y931C and E864K—that decreased the potency of multiple JAK2 inhibitors in cellular assay systems. The authors go on to report an ability of heat shock protein 90 (HSP90) inhibitors to circumvent the potency shift observed with JAK inhibition.[4] JAK2 inhibitors were less potent when these in vitro-generated synthetic residue substitutions were present in cis with clinically relevant somatic-activating JAK2 mutations, i.e., JAK2 V617F, which is characteristic of MPNs,[5] and JAK2 R683G, which is found in a subset of individuals with B-cell acute lymphoblastic leukemia (B-ALL) with rearrangements of cytokine receptor-like factor 2 (CRLF2).[6] Structural modeling studies determined that the synthetic G935R, Y931C and E864K amino acid changes were located near the JAK2 ATP binding site, which led to the hypothesis that they would interfere with JAK2 inhibitor binding.[4]

The in vitro experimental process yielded G935R, Y931C and E864K by exposure of CRLF2-expressing murine Ba/F3 cells transduced with synthetically altered human JAK2 R683G cDNA to high concentrations of the JAK2 inhibitor NVP-BVB808. These JAK2 variants also reduced the responsiveness of erythropoietin receptor (EpoR)-expressing Ba/F3 cells to this JAK inhibitor. Using similar in vitro methods, others have also identified these JAK2 alterations,[7-9] though it is noteworthy that they have not been reported in either JAK2 V617F-driven mouse models of MPN-like diseases following treatment with JAK inhibitors or in patients. Testing of a panel of JAK2 inhibitors against the mutant

EpoR-expressing Ba/F3 cells transduced with mouse JAK2 V617F revealed that G935R and Y931C decreased the potency of ruxolitinib in this system. Of note, the concentration of ruxolitinib required to inhibit cell growth by 50% (GI_{50}) increased approximately 3-fold in the presence of the G935R mutation and 9-fold with the Y931C mutation.[4]

Because JAK2 is an HSP90 client,[10] and inhibition of HSP90 results in wild-type and mutant JAK2 depletion,[11] HSP90 inhibitors were also evaluated in these in vitro-generated JAK2 mutant clones. In this report by Weigert et al., addition of HSP90 inhibitors led to frank cytotoxicity rather than growth inhibition because of cell cycle accumulation in G_1 or G_2, which is typically seen in other experimental settings with HSP90 inhibitors.[12] This cytotoxic effect led the authors to suggest that HSP90 inhibition may be mechanistically relevant in overcoming JAK2 inhibitor resistance (**Fig. 1**). However, these findings also suggest that HSP90 inhibition is likely a less selective approach than direct inhibition of JAK2. Indeed, HSP90 has numerous client proteins in addition to JAK2, and HSP90 inhibitors have shown cytotoxic activity in a great variety of in vitro malignancy-derived cell lines in addition to strictly JAK2-dependent cell lines. Given the above, the authors acknowledged the possibility that interference of HSP90

inhibitors with signaling pathways not involving JAK2 contributed to cell kill. In nude mice transplanted with Ba/F3 cells containing the Y931C mutation, treatment with the HSP90 inhibitor NVP-AUY922 improved overall survival compared with vehicle; however, the effects of NVP-BVB808 were not evaluated in this setting and the tolerability to NVP-AUY922 was not described. In *CRLF2*-rearranged B-ALL xenografts established from the bone marrow of B-ALL patients and implanted into mice, NVP-AUY922 was more efficacious than NVP-BVB808 at suppressing JAK-STAT, MAP kinase and AKT signaling and was associated with prolonged survival compared with NVP-BVB808. However, one should recognize that these xenografts lacked any secondary JAK2 mutations that would confer resistance to JAK inhibition.[4] Moreover, the dose of NVP-BVB808 used in this experiment was insufficient to produce any noteworthy pharmacodynamic suppression of either pJAK2 or pSTAT.

Although the resistance observed with JAK inhibitors in this study is intriguing, there are qualitative and quantitative differences between the resistance described by Weigert et al. and that described for BCR-ABL inhibitors, such as imatinib, or the more potent second-generation inhibitor nilotinib. Most significant is the lack (to date) of documented clinical resistance

due to the emergence of JAK2 secondary mutations in patients treated with JAK inhibitors. While this may be the result of a less pronounced oncogenic role for JAK2 signaling in MF patients, it is intriguing, yet perhaps coincidental, that all three publications describing JAK2 variants with reduced sensitivity to JAK inhibitors utilized the expression of unnatural secondary JAK2 mutations rather than relying on natural selection. Quantitatively, the mutations described by Weigert et al. conveyed a modest degree ($<$ 4-fold to 9-fold) of resistance relative to the wild-type protein. This is in contrast to those mutations in BCR-ABL identified by Ray et al. in a similar model system. In that study, five clinically occurring BCR-ABL mutations conveyed a degree of resistance to the second-generation inhibitor nilotinib greater than 20-fold with at least two BCR-ABL mutants conveying greater than 100-fold resistance over wild-type BCR-ABL.[13]

What are the implications of this study for patients being treated with ruxolitinib? While the findings are of interest, their clinical relevance remains to be determined. It is not known whether patients treated with JAK2 inhibitors develop G935R, Y931C or E864K mutations during treatment, as these variants have not been identified in the clinic. Additionally, a clinical definition of resistance to JAK
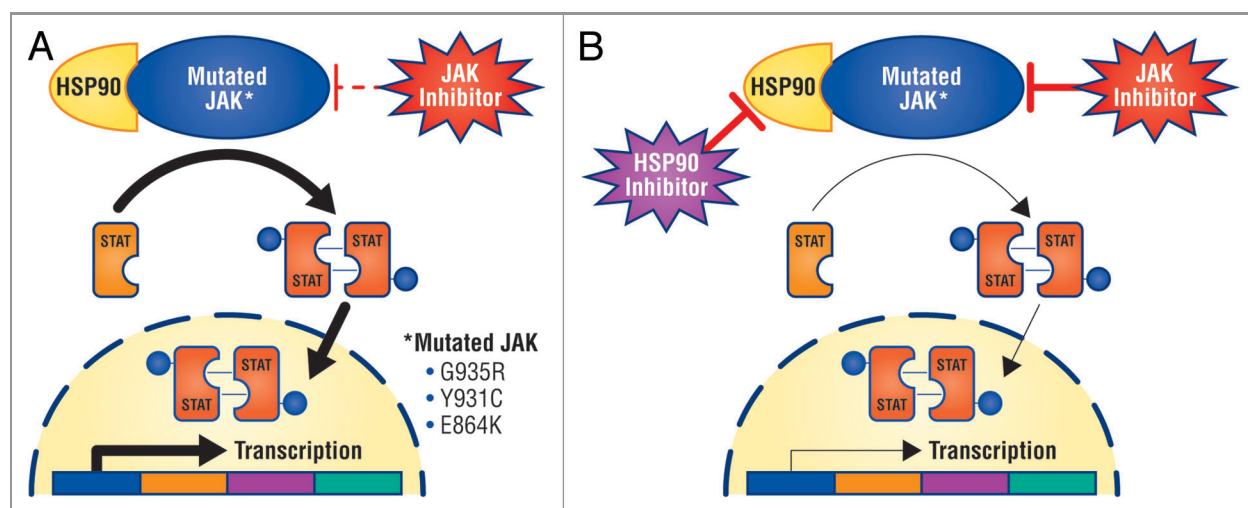

Figure 1. A model based on the findings of the study by Weigert et al. would imply that in the presence of certain JAK2 mutations, JAK2 inhibitors may be unable to maximally inhibit JAK-STAT signaling (A). In this model, the co-administration of an HSP90 inhibitor could supplement the effect of a JAK2 inhibitor to overcome compromised JAK2 inhibitor potency (B).

inhibitors in the setting of MPNs, and specifically MF, has not been published. Indeed, long-term follow-up of patients treated with JAK2 (or JAK1/JAK2) inhibitors to determine whether they develop resistance to such agents, and if so elucidation of the mechanisms of resistance, will be necessary to understand whether the G935R, Y931C and E864K amino acid changes are clinically meaningful. Perhaps in clinical situations where the selective pressure for spontaneous emergence of JAK2 mutations is higher (e.g., acute leukemia), true "resistance mutations" may be more readily selected. Lastly, the lack of specificity of HSP90 inhibitors with regard to molecular moieties or pathways whose activity would secondarily be influenced by these agents may result in tolerability issues. The clinical utility of HSP90 inhibitors in diseases in which JAK-STAT inhibition is (or may become) of therapeutic value will require further study.[10] Indeed, while clinical studies exploring the combination of HSP90 inhibitors and a JAK inhibitor may be the most efficient way to translate the preclinical work of Weigert et al., it will be equally important to explore the single-agent antineoplastic efficacy of HSP90 inhibitors, interrogate whether HSP90 system-related biology underlies differences in the magnitude of clinical benefit of JAK inhibitors and characterize emerging mechanisms of resistance to the latter class of agents.

References

1. Press release. FDA approves Incyte's Jakafi™ (ruxolitinib) for patients with myelofibrosis. 11/16/11.
2. JAKAFI™ (ruxolitinib) prescribing information. Incyte Corporation, Wilmington, DE. 2011.
3. ClinicalTrials.gov. Available at: http://clinicaltrials.gov/ct2/results?term=ruxolitinib. Accessed 3/2/12.
4. Weigert O, Lane AA, Bird L, Kopp N, Chapuy B, van Bodegom D, et al. Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition. J Exp Med 2012; 209:259-73; PMID:22271575; http://dx.doi.org/10.1084/jem.20111694
5. Jatiani SS, Baker SJ, Silverman LR, Reddy EP. Jak/STAT pathways in cytokine signaling and myeloproliferative disorders: approaches for targeted therapies. Genes Cancer 2010; 1:979-93; PMID:21442038; http://dx.doi.org/10.1177/1947601910397187
6. Yoda A, Yoda Y, Chiaretti S, Bar-Natan M, Mani K, Rodig SJ, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A 2010; 107:252-7; PMID:20018760; http://dx.doi.org/10.1073/pnas.0911726107

7. Hornakova T, Springuel L, Devreux J, Dusa A, Constantinescu SN, Knoops L, et al. Oncogenic JAK1 and JAK2-activating mutations resistant to ATP-competitive inhibitors. Haematologica 2011; 96:845-53; PMID:21393331; http://dx.doi.org/10.3324/haematol.2010.036350
8. Marit M, Chohan M, Matthew N, Huang K, Barber D. Random mutagenesis reveals residues of JAK2 critical in evading inhibition by tyrosine kinase inhibitors. Blood (ASH Annual Meeting Abstracts) 2008; 112:3717.
9. Deshpande A, Reddy MM, Schade GOM, Ray A, Chowdary TK, Griffin JD, et al. Kinase domain mutations confer resistance to novel inhibitors targeting JAK2V617F in myeloproliferative neoplasms. Leukemia 2012; 26:708-15; PMID:21926964; http://dx.doi.org/10.1038/leu.2011.255
10. Marubayashi SP, Koppikar P, Taldone T, Abdel-Wahab O, West N, Bhagwat N, et al. HSP90 is a therapeutic target in JAK2-dependent myeloproliferative neoplasms in mice and humans. J Clin Invest 2010; 120:3578-93; PMID:20852385; http://dx.doi.org/10.1172/JCI42442

11. Proia DA, Foley KP, Korbut T, Sang J, Smith D, Bates RC, et al. Multifaceted intervention by the Hsp90 inhibitor ganetespib (STA-9090) in cancer cells with activated JAK/STAT signaling. PLoS One 2011; 6: e18552; PMID:21533169; http://dx.doi.org/10.1371/journal.pone.0018552
12. Yamaki H, Nakajima M, Shimotohno KW, Tanaka N. Molecular basis for the actions of Hsp90 inhibitors and cancer therapy. J Antibiot (Tokyo) 2011; 64:635-44; PMID:21811259; http://dx.doi.org/10.1038/ja.2011.60
13. Ray A, Cowan-Jacob SW, Manley PW, Mestan J, Griffin JD. Identification of BCR-ABL point mutations conferring resistance to the Abl kinase inhibitor AMN107 (nilotinib) by a random mutagenesis study. Blood 2007; 109:5011-5; PMID:17303698; http://dx.doi.org/10.1182/blood-2006-01-015347