

Resistance to tyrosine kinase inhibitors reversed by selective JAK inhibition

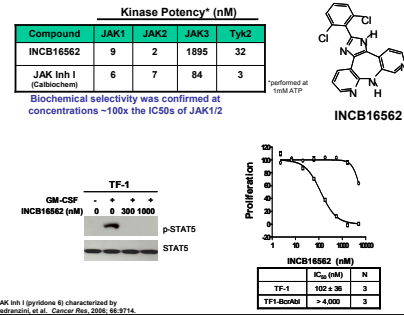
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ABSTRACT #4727

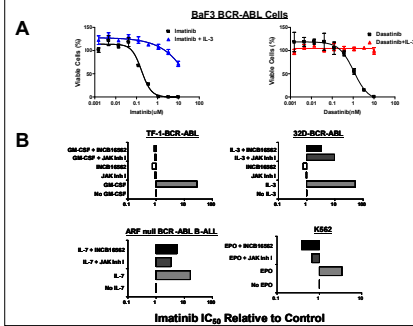
Tyrosine kinase inhibitors (TKIs) such as imatinib have demonstrated remarkable benefit, yet relapse due to drug resistance has limited their success. Secondary kinase mutations are one mechanism of resistance, still additional sources exist. The cytokine and growth factor rich milieu of cancer has led to the hypothesis that cancer cells may evade the effects of therapy using alternative survival pathways. Many of these factors signal through JAK kinases thereby activating downstream survival pathways, such as the STAT family of transcription factors. We hypothesized that JAK activation would convey resistance to TKIs and that JAK inhibition would restore responsiveness to therapy.

BaF3 cells were transformed via oncogenic tyrosine kinase expression (EGFR, TPR-MET, or BCR-ABL). Selective TKIs were used to determine their effects on proliferation and cell signaling in the presence or absence of IL-3, a JAK-activating cytokine. In each case, JAK activation rendered cells resistant to the cytoreductive effects of TKIs (2-fold to ≥ 100 -fold), but not to their effects on cell signaling. Similar trends were seen using other JAK-activating factors such as GM-CSF or Epo in BCR-ABL expressing TF-1 or K562 cells, respectively. Importantly, treatment of cytokine-stimulated cell lines with a selective JAK inhibitor - JAK Inhibitor I (Cabotchem) or INCB16562 - shifted the IC50 for the respective TKI to near control levels. JAK inhibition by itself had limited effects; moreover, the consequences of JAK activation or inhibition were drastically muted when EGFR or BCR-ABL were mutated at their respective "gate-keeper" residues rendering them resistant to TKI binding. This suggests the resistance and re-sensitization described above is specifically due to JAK activation and inhibition, respectively. Indeed, expression of constitutively active JAK1V658F, but not the unphosphorylated STAT3 dimers conveyed resistance to imatinib in BCR-ABL expressing cells. These data indicate that JAK activation is sufficient to provide resistance to TKIs and that dimerization of STAT3 is not, implying STAT phosphorylation by JAKs is required. *In vivo*, we utilized a model of BCR-ABL+ ALL developed by Williams, et al. who demonstrated a protective role for IL-7 that was mitigated by JAK inhibition *in vitro*. Inoculation of these transformed B-cell results in mortality at ~ 3 weeks. Treatment with the ABL TKI dasatinib modestly improved survival ($\sim 30\%$, $p < 0.05$), consistent with Williams' identification of a protective role for γc cytokines *in vivo*. However, the addition of a JAK inhibitor increased the median survival time by $> 120\%$ ($p < 0.01$) while having no significant effect by itself ($p > 0.05$). These data demonstrate that JAK activation conveys resistance to a broad range of clinically relevant TKIs and that pharmacological inhibition of JAKs can mitigate these effects resulting in a favorable therapeutic response thus warranting clinical evaluation.

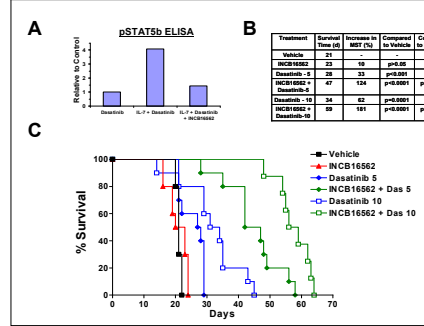
1. Biochemical and cellular characterization of Incyte JAK inhibitors



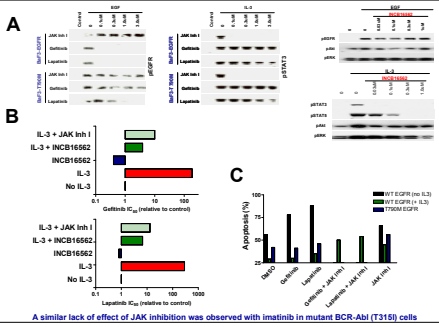
2. JAK activating cytokines confer resistance to Abl TKIs (A&B) - restoration of sensitivity by selective JAK inhibitors (B)



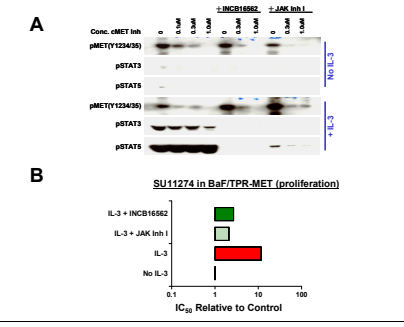
3. JAK inhibition antagonizes cytokine-mediated STAT5 phosphorylation (A) and improves survival (B & C) in a murine BCR-ABL+ ALL model



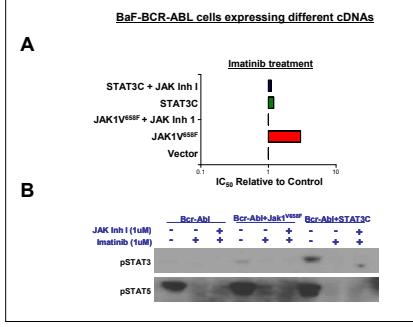
4. JAK1/2 inhibition reverses IL-3 mediated resistance to EGFR inhibitors (B & C) with limited effect on resistance mediated by the EGFR T790M gatekeeper mutation (C)



5. The efficacy of c-Met inhibition is antagonized by JAK activating cytokines



6. Expression of activated JAK (JAK1V658F) is sufficient to cause resistance to TKIs while dimerization of STAT3 is not



CONCLUSIONS

- Clinical resistance to TKIs are not invariably associated with kinase domain mutations
- JAK activation, through cytokines or mutation, conveys resistance to multiple clinically relevant TKIs
- Selective inhibition of JAK1/2 restores sensitivity to imatinib, dasatinib, gefitinib, lapatinib, and the c-Met inhibitor SU11274 *in vitro*
- The combination of a JAK1/2 inhibitor and an Abl TKI results in significantly improved survival in a murine model of B-ALL *in vivo*
- Because aberrant JAK/STAT activation and elevated cytokine levels are frequently observed in cancer patients, investigation of regimens combining selective JAK1/2 inhibitors with other TKIs is warranted

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