Pacritinib Reduces Human Myeloid Leukemia Stem Cell Maintenance in a Defined Niche

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Background

Pacritinib, a potent clinical small molecule inhibitor of JAK2, but not JAK1 that does not cause marrow suppression and has demonstrated efficacy in myelofibrosis. Pacritinib also suppresses signaling through wild-type and mutant FLT3, IRAK1, and CSF1R at less than 50n. Pacritibnib has demonstrated single agent activity preclinically in other myeloid neoplasms including AML and CMML. Stromal protection was not observed. However, the capacity of pacritinib to eradicate therapy resistant leukemia stem cells (LSC), residing in the bone marrow niche, had not been examined. Thus, we investigated the impact of pacritinib alone or in combination with standard of care therapy on primary blast crisis chronic myeloid leukemia (BC CML), myelofibrosis (MF) and

Pacritinib (SB1518) JAK2/FLT3inhibitor : Unique Kinome Profile

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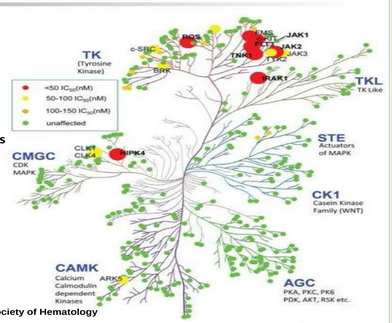
- Pacritinib is an oral multikinase inhibitor with specificity for kinases in several unrelated families
- The inhibition of IRAK1 and c-fms (CSF1R) may contribute to its anti-inflammatory activity and early onset of symptomatic benefit in MF
- Suggests its potential therapeutic utility in AML, MDS and, particularly CMML, CLL due to its potent inhibition of c-fms, IRAK1, JAK2 and FLT3.

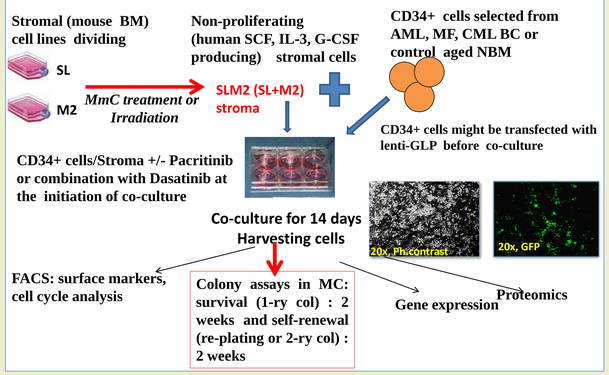


Singer J, et al., Abstract #1874 American Society of Hematology Annual Meeting 2014.

Materials & Methods

Genetically engineered mouse bone marrow fibroblasts producing human SCF, IL3 and G-CSF were used as stromal monolayers to support LSC survival and self-renewal. Human primary CD34+ cells were selected from BC CML (n=10), MF (n=10) and relapsed AML (n=4) before and after clinical treatment with azacitidine (Vidaza). As a control, CD34+ cells from age matched normal bone marrow (a-NBM, n=4) were used for the co-culture. Survival and self-renewal of the cells were investigated by colony forming and replating assays. Pacritinib was used at concentrations ranging from 10 to 50 nM alone and in combination with 1 nM dasatinib.

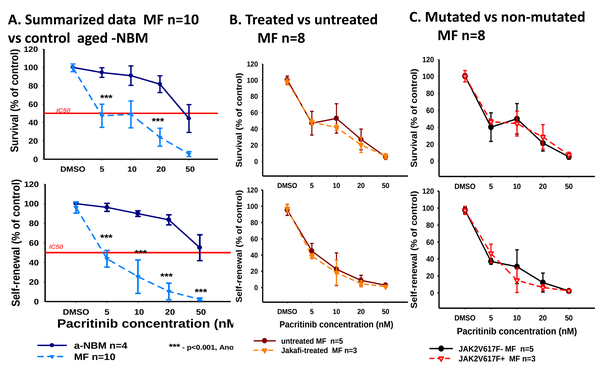
In vitro model of Pacritinib treatement of leukemia progenitors in the presence of the supporting niche



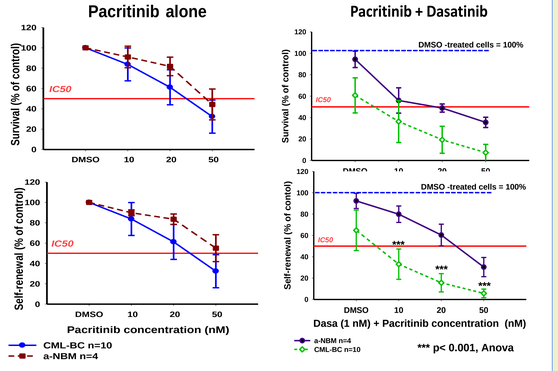
Results

Pacritinib alone induced dose-dependent inhibition of survival and self-renewal in a-NBM, AML, MF and CML-BC with high doses (50nM) being cytotoxic. At the optimal concentration of 20nM pacritinib demonstrated the possible diversity in reaching the IC50 between normal and leukemia progenitors. Aged-NBM as well as AML and MF cells responded uniformly with no significant differences between samples, and inhibition reached 50% at 10nM concentration. BC CML were more divergent in their reaction to the compound: 2 out of 5 (40%) samples demonstrated high (>50%) inhibition, in another 2(40%) samples it was intermediate (20-50%) and in 1 sample (20%) inhibition was low (<20%). Notably, there was no correlation between the presence of JAK2 mutation in the MF samples and their response to pacritinib exposure.
Combined treatment with the low dose of dasatinib (1 nM) and pacritinib doses of 10 or 20 nM resulted in a statistically significant ($p<0.001$, Anova) difference in survival and self-renewal of BC CML compared with normal progenitors. Importantly, even those CML samples with intermediate and low response demonstrated decreased ability of survival and self-renewal, raising a possibility of an additive/synergistic mechanisms. Progenitors from AML samples collected before and after clinical treatment with azacitidine uniformly showed a significant decrease in survival and self-renewal starting with 10 nM pacritinib alone. Combined treatment of AML samples (both pre- and post- azacitidine) with dasatinib did not enhance the inhibitory effect of pacritinib on self- renewal, suggesting a prospect of using the compound as a single agent in the treatment of relapsed AML.

Pacritinib inhibits survival and self-renewal of MF (n=10) in dose dependent manner independent of previous treatment or Jak2 mutation status

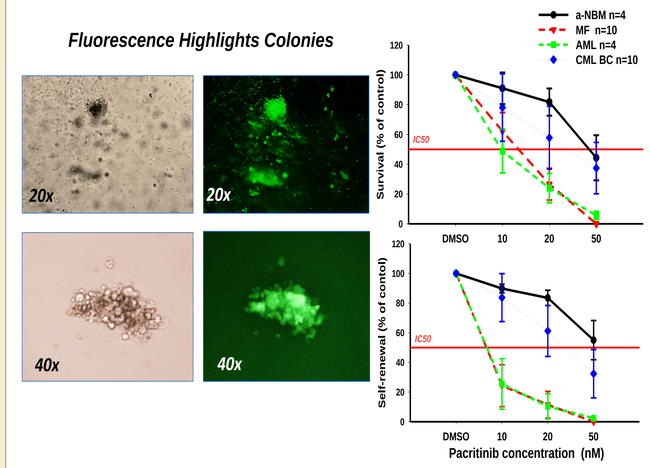


Dasatinib enhanced Pacritinib-mediated eradication of self-renewing LCS in CML BC (n=10)

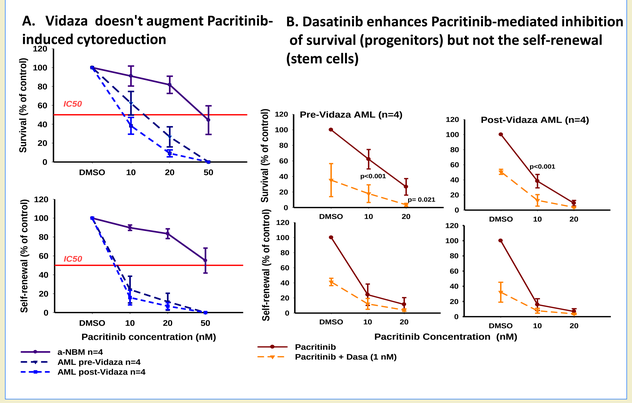


Summarized comparative results for all tested samples

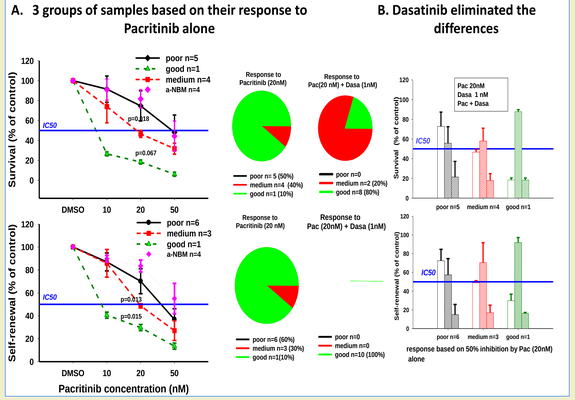
Lenti-GLF leukemia colony-formation after 2 weeks SLM2 co-culture



Vidaza and Dasatinib do not enhance the inhibitory effect of Pacritinib in AML (n=4)



Combined treatment of CML BC with Paritinib and Dasatinib



Conclusions

- Pacritinib alone, possibly through inhibition of CSF1 and IRAK1 signaling in addition to suppression of JAK2, at readily clinically achievable low nM concentrations is effective in reducing survival and self-renewal in relapsed AML and MF even in the presence of a LSC supportive niche.
- In CML BC a combination of dasatinib and pacritinib is required to eliminate self-renewing LSC, with minimal toxicity toward normal progenitors.
- Targeting niche-dependent signaling could represent a robust avenue for treatment of refractory myeloid leukemia.

There is nothing to disclose