**Background**

Pacritinib, a potent clinical small molecule inhibitor of JAK2, has not been identified that does not cause major adverse events. Pacritinib and clinical biomarkers are not always predictive of therapeutic response. Agnostic with previously classiﬁed genetic abnormalities and existing biomarkers is required. This research presents an in vitro and in vivo investigation of the utility of Pacritinib as an anti-leukemic agent and identiﬁed the supportive niche in AML, MDS, and CML-BC.

**Materials & Methods**

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 doubling in reaching the IC50 between normal and leukaemic progenitors. Aged-NBM as well as AML and MF cells responded uniformly with no signiﬁcant difference between samples, and inhibition reached 50% at 50nM concentration. BC CML were more divergent in their reaction to the compound. 2 out of 5 (40%) samples demonstrated high (≥90%) inhibition, in another (20%) samples it was intermediate (50-70%) and in 1 sample (20%) inhibition was low (≤50%). Notably, there was no correlation between IC50 values and the major biomarkers (JAK2 V617F or BCR-ABL). Controled treatment with the low dose of dasatinib (1nM) and pacritinib doses of 5 or 10 nM resulted in a statistically signiﬁcant (p=0.01) anomaly difference in survival and self-renewal of BC CML compared with normal progenitors. Importantly, even these CML samples with intermediate low ﬁne demonstrated decreased ability of survival and self-renewal, making a possibility of an additional synergistic mechanism. 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 prosop of using the compound as a single agent in the treatment of relapsed AML.

**Results**

**Conclusions**

- Pacritinib, possibly through inhibition of CSF1 and IRAK signaling in addition to suppression of JAK2, markedly clinically susceptible low IC50 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 limited toxicity towards normal progenitors.
- Targeting niche-dependent signalling could represent a robust avenue for treatment of refractory myeloid leukemias.