Pacritinib Blocks Key Pro-survival Signaling Related to Mutated MYD88, Produces High Levels of Apoptosis and Overcomes Mutated BTK^{Cys481} Related BTK-inhibitor Resistance

S Liu (1, 2), A Kofides (1), X Liu (1), A Canning (1), N Tsakmaklis (1), M Guerrera (1, 2), C. Patterson (1), Z Hunter (1, 2), K Richardson (1), C Flynn (1), K Meid (1), M Little (1), K Stockman (1), T White (1), S Sarosiek (1, 2), J Castillo (1, 2), and S Treon (1, 2).

(1) Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA

(2) Department of Medicine, Harvard Medical School, Boston, MA, USA.

INTRODUCTION

Somatic mutations in MYD88 are prevalent in many B-cell malignancies, including Waldenström Macroglobulinemia (WM; 95-97%), primary CNS lymphoma (70-80%), ABC DLBCL (40%), marginal zone lymphoma (5-10%) and CLL (5-15%) (1,2). Mutated MYD88 drives oncogenic pro-survival signaling through multiple signaling cascades that include HCK-directed BTK, ERK and SYK, as well as IRAK1/IRAK4 activation (3-5). HCK is an SRC family member that mutated MYD88 transcriptionally upregulates, and its activation is triggered by IL-6/IL-6R/gp130/JAK2 signaling. BTK inhibitors are active in MYD88 mutated B-cell malignancies, but fail to produce complete responses, and show varying durations of response that are disease dependent and impacted by depth of response (3-5). Acquired resistance to BTK inhibitors is commonly related to a BTK^{Cys481} mutation (6). Pacritinib is a kinase inhibitor, FDA-approved for myelofibrosis, that is well tolerated and targets pro-signaling cascades that are relevant to mutated MYD88 signaling, including IRAK1, JAK2, and SRC, a homologue of HCK (7,8) (**Fig. 1**). Pacritinib may therefore represent a more effective option in blocking mutated MYD88 pro-survival signaling versus BTK-inhibitors.

AIM

To evaluate the therapeutic effect of pacritinib for malignancies related to mutated MYD88

METHOD

We performed comparative studies to evaluate the activity of pacritinib and the covalent BTK-inhibitors ibrutinib and zanubrutinib on mutated MYD88 relevant pro-survival signaling, as well as proliferation and survival in MYD88 mutated cell lines and primary MYD88-mutated WM cells. Further to these studies, we assessed the activity of pacritinib in well-characterized mutant (BTK^{Cys481Ser}) expressing covalent BTK-inhibitor resistant WM and ABC DLBCL lymphoma cell models.

RESULTS

The apoptotic activity of pacritinib showed dose dependence with high levels of apoptosis at 0.5 μM (**Fig. 2**), which is well within its pharmacologically achievable levels. In addition to its known effects on JAK2 and IRAK1 activation, pacritinib showed robust inhibition of p-HCK (Y411) and its downstream signaling partner p-BTK (Y223) in MYD88 mutated BCWM.1 and TMD-8 cells (**Fig. 3**). Importantly, pacritinib alone and combined with venetoclax induced high levels of apoptosis in BTKCys481Ser expressing covalent BTK-inhibitor resistant MYD88 mutated WM and ABC DLBCL lymphoma cells (**Fig. 4**). Lastly, We treatment with pacritinib alone induced roburst apoptosis in the primary malignant cells of WM bone marrow (**Fig. 5**).

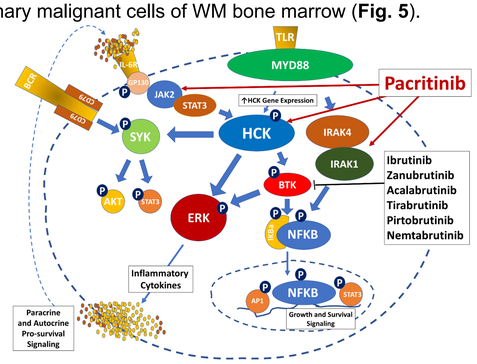

Figure 1. Pro-survival signaling mediated by mutated MYD88. Targets for therapeutic intervention by pacritinib and BTK-inhibitors are identified.

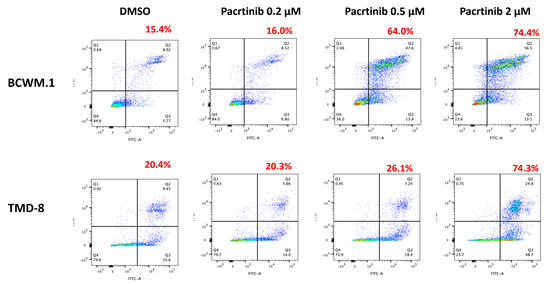

Figure 2. Pacritinib induces apoptosis/death of BCWM.1 and TMD-8 Cells. BCWM.1 cells and TMD-8 cells were treated with various concentration of pacritinib for 20 hours. Induced cell death and apoptosis were evaluated by Flow Cytometry with Annexin V-FITC and PI Staining.

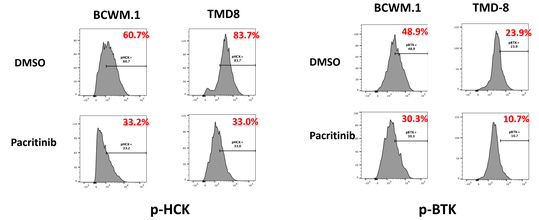

Figure 3. Pacritinib treatment significantly decreases the phosphorylation of HCK and BTK in BCWM.1 and TMD8 cells. BCWM.1 cells and TMD-8 cells were treated with 0.5 μM pacritinib for 2 hours. Effects on the phosphorylation of HCK and BTK were measured by Flow Cytometry with Phosflow™ staining using conjugated monoclonal antibodies for HCK (Tyr411) (clone E5L3D) and BTK (pY223)/Itk (pY180) (clone N35-86).

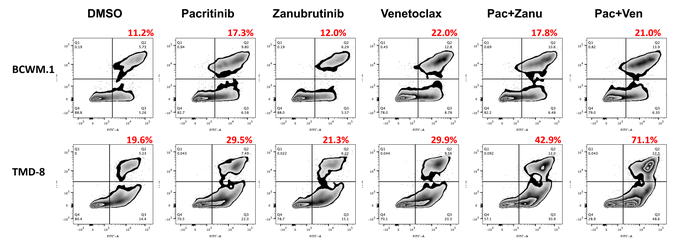

Figure 4. Effect of Pacritinib treatment on the apoptosis of BTK^{Cys481Ser} mutated BCWM.1 and TMD-8 cells: A combination with Pacritinib overcomes resistance to BTKi. BTK^{Cys481Ser} –overexpressing BCWM.1 cells and TMD-8 cells, which are constitutively active for BTK, were treated with 0.5 μM of pacritinib, zanubrutinib, venetoclax alone, or in combination for 20 hours. Induced cell death and apoptosis were evaluated by Flow Cytometry with Annexin V-FITC and PI Staining

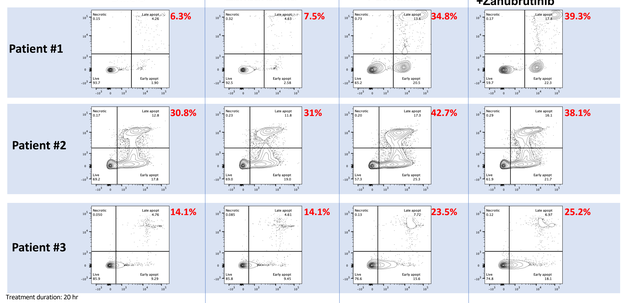

Figure 5. Pacritinib Induces Apoptosis/death of CD19+ cells in the Untreated WM Patient BMMCs. Human bone marrow mononuclear cells from untreated 3 WM patients were treated with 0.5 μM of pacritinib, zanubrutinib, alone, or in combination for 20 hours. Induced cell death and apoptosis were evaluated by Flow Cytometry with Annexin V-FITC and PI Staining

CONCLUSIONS

Pacritinib more broadly extinguishes mutated MYD88 pro-survival signaling cascades (Fig 1.) and demonstrates high levels of apoptotic activity in mutated MYD88 WM and ABC DLBCL cells versus selective covalent BTK-inhibitors. Pacritinib also synergizes with covalent BTK- and BCL2 inhibitors and can overcome covalent BTK-inhibitor resistance related to mutated BTKCys481. Pacritinib demonstrates higher levels of apoptotic activity for patient bone marrow malignant B cells. Our studies provide a framework for investigating pacritinib in MYD88 mutated lymphomas. A clinical trial of pacritinib in relapsed/refractory WM is being initiated.

REFERENCES

1. *Treon SP. et al. MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia. N. Engl. J. Med. 367, 826–833 (2012).*
2. *Ngo, V. N. et al. Oncogenically active MYD88 mutations in human lymphoma. Nature 470, 115–119 (2011).*
3. *Yang, G. et al. HCK is a survival determinant transactivated by mutated MYD88, and a direct target of ibrutinib. Blood 127, 3237–3252 (2016).*
4. *Liu, X. et al. Expression of the prosurvival kinase HCK requires PAX5 and mutated MYD88 signaling in MYD88-driven B-cell lymphomas. Blood Adv 4, 141–153 (2020).*
5. *Munshi, M. et al. A new role for the SRC family kinase HCK as a driver of SYK activation in MYD88 mutated lymphomas. Blood Adv 6, 3332–3338 (2022).*
6. *Chen, J. G. et al. BTKCys481Ser drives ibrutinib resistance via ERK1/2 and protects BTKwild-type MYD88-mutated cells by a paracrine mechanism. Blood 131, 2047–2059 (2018).*
7. *Singer, J. W. et al. Comprehensive kinase profile of pacritinib, a nonmyelosuppressive Janus kinase 2 inhibitor. J Exp Pharmacol **8**, 11–19 (2016).*
8. *Venugopal, S. & Mascarenhas, J. The Odyssey of Pacritinib in Myelofibrosis. Blood Adv. 6, 4905–4913 (2022).*

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the generous support of Peter Bing M.D., International Waldenstrom's Macroglobulinemia Foundation, Leukemia and Lymphoma Society (Grant: 6673-24), NIH SPORE in Multiple Myeloma (Grant: 2P50CA100707-16A1), the Orszag and Kitchen Fund for WM Research, the Kerry Robertson Fund for WM Research, the Siegel Family Fund for WM, the WM Foundation of Canada, the WMR Fund, Lee and Michael Bell WM Fellowship, and the WM patients who provided samples for these studies.

CONTACT INFORMATION

Shirong Liu, MD, PhD Email: shirong_liu@dfci.Harvard.edu