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Poster P991

Adding Navtemadlin to Ruxolitinib Potentiates Apoptosis in Myeloblasts From Patients With Myelofibrosis

Tracy Clevenger*[1], Jean Cheung*[1], Fanny Krantz[1], Michael Gulrajani[1], Cynthia Nguyen[1], Miranda Luarca[1], Cecile Krejsa[1], Wayne Rothbaum[1], Todd Covey[1]

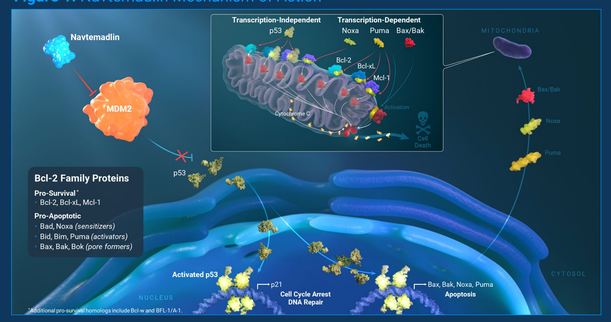
[1]Kartos Therapeutics, Inc., Redwood City, CA, USA
*Equal contributions

For more information, please contact:
Tracy Clevenger: tclevenger@kartosthera.com
Jean Cheung: jcheung@kartosthera.com
Todd Covey: tcovey@kartosthera.com

Background

- Ruxolitinib, a Janus kinase 1/2 inhibitor, can improve splenomegaly and myelofibrosis (MF)-related symptoms; most patients on ruxolitinib experience a suboptimal response that results in treatment discontinuation[1] over time
- Navtemadlin, a potent, selective, orally available mouse double minute 2 (MDM2) inhibitor restores tumor protein 53 (p53) function, modulates B-cell lymphoma 2 (Bcl-2) family proteins and drives apoptosis of wild-type (*TP53*^{WT}) myeloblasts[2,3] (Figure 1)
- Single agent navtemadlin demonstrated clinically meaningful and disease modifying activity in *TP53*^{WT} relapsed/refractory MF[4,5]
- Efficient MDM2i-driven apoptosis requires overcoming the anti-apoptotic checkpoint cyclin-dependent kinase inhibitor 1 (p21), a critical regulator of cell cycle arrest[6]
- We reasoned combining navtemadlin with ruxolitinib may have synergistic potential to inhibit p21-mediated cell cycle arrest and block dysregulated Bcl-2 signaling mechanisms used by MF cells to escape cell death

Figure 1. Navtemadlin Mechanism of Action



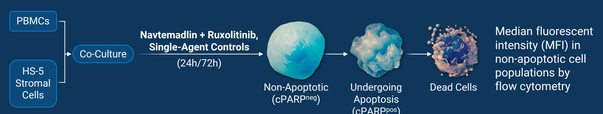
Objective

To assess the impact of adding navtemadlin to ruxolitinib in MF and blast-phase myeloproliferative neoplasm (MPN-BP) cell lines and patient samples

Methods

- UKE-1, a *TP53*^{WT} MF cell line, was used for drug-sensitivity testing and assessing single agent navtemadlin, ruxolitinib or synergy of the combination:
  - Drug-sensitivity was performed using multi-parameter intracellular flow cytometry
  - Proliferation was measured by CellTiter-Glo® luminescent assay (Promega)
  - Percent of control was calculated and synergy was assessed using Combenefit software, HSA synergy model
- Drug-sensitivity profiling was performed on peripheral blood mononuclear cells (PBMCs) from MF (n=15) and MPN-BP (n=17) patient samples
- PBMCs were co-cultured with HS-5 stromal cells at clinically relevant concentrations of navtemadlin (3μM) and ruxolitinib (0.25-1μM) for 24h or 72h (Figure 2)
- Antibody panels were used to:
  - Detect apoptosis (i.e., cPARP)
  - Measure p21, p53 and Bcl-2 family proteins in non-apoptotic cell subsets
- Statistics were performed using Dunnett's test

Figure 2: Co-Culture of PBMCs

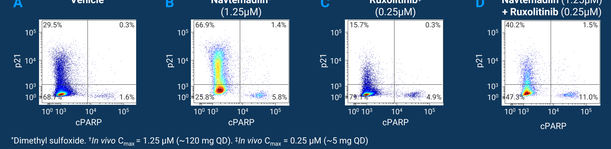


Results

UKE-1 Cell Line

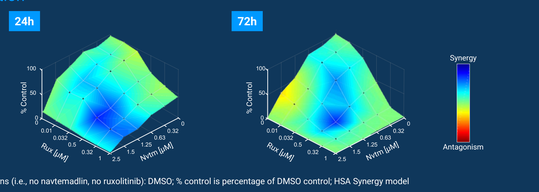
- Control conditions are shown in Figure 3A
- Navtemadlin increased p21 levels and induced apoptosis (Figure 3B); ruxolitinib had no affect on p21 levels and showed modest cell-killing (Figure 3C)
- Ruxolitinib potentiated the cytotoxicity of navtemadlin and inhibited p21 compared with navtemadlin alone (Figures 3B and 3D)
- Navtemadlin combined with ruxolitinib was synergistic (Figure 4)
- Ruxolitinib enhanced navtemadlin-driven apoptosis with near-complete cell-killing observed at 72h (Figures 5A-B) and decreased induction of p21 (Figures 5C-D) and p53 (Figures 5E-F)
- Only cells without p21 expression were apoptotic, suggesting ruxolitinib inhibited the protective role of p21 in p53-driven apoptosis

Figure 3. Intracellular Flow Cytometry of UKE-1 Cells: 24h Exposure to Navtemadlin, Ruxolitinib or the Combination



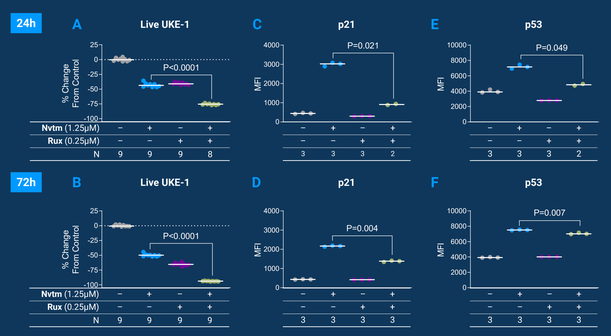
*Dimethyl sulfoxide. †*In vivo* C_{max} = 1.25 μM (~120 mg QD). ‡*In vivo* C_{max} = 0.25 μM (~5 mg QD)

Figure 4. Navtemadlin Combined With Ruxolitinib: Synergistic Impacts on UKE-1 Cell Proliferation



Control conditions (i.e., no navtemadlin, no ruxolitinib): DMSO; % control is percentage of DMSO control; HSA Synergy model

Figure 5. Cell Survival and Protein Expression in UKE-1 Cells: 24h or 72h Exposure to Navtemadlin, Ruxolitinib or the Combination



Control conditions (i.e., no navtemadlin, no ruxolitinib): DMSO
% change from control calculated with % cPARP^{neg} cells of all cells; MFI determined from non-apoptotic cells

Abbreviations
Bcl-2, B-cell lymphoma 2 inhibitor; Bcl-xL, B-cell lymphoma-extra large inhibitor; C_{max}, maximum serum concentration; cPARP, cleaved PARP, cleaved poly(ADP-ribose) polymerase; DMSO, dimethyl sulfoxide; h, hour; HSA, Highest Single Agent; μM, micromolar; Mcl-1, induced myeloid leukemia cell differentiation protein; MF, myelofibrosis; MFI, median fluorescence intensity; MPN-BP, myeloproliferative neoplasm-blast phase disease; ns, not significant; Nvtm, navtemadlin; p21, cyclin-dependent kinase inhibitor 1; p53, tumor protein 53; pts, patients; QD, once-a-day; Rux, ruxolitinib.

References
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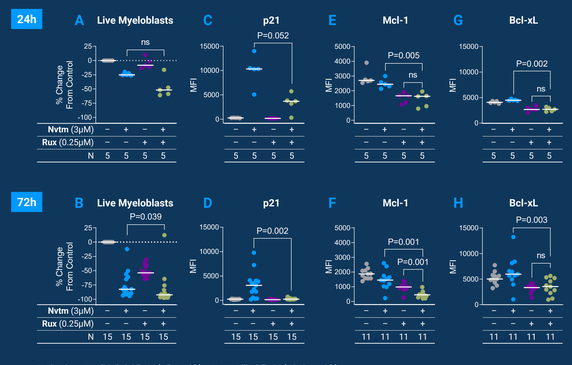
Myeloblasts From Patients

- In MF cells cultured for 24h or 72h, navtemadlin added to ruxolitinib increased apoptosis of myeloblasts (Figures 6A-B) compared with navtemadlin alone (P=0.039, Figure 6B)
- Prior to the start of apoptosis, navtemadlin-mediated induction of p21 (Figures 6C-D) was significantly reduced with the combination (P=0.002, Figure 6D) compared with navtemadlin alone
- The combination treatment also reduced pro-survival Bcl-2 family protein Mcl-1 expression (Figures 6E-H), with near-complete inhibition of expression at 72h compared with navtemadlin or ruxolitinib alone (P=0.001, Figure 6E)
- Ruxolitinib decreased Bcl-xL levels, while navtemadlin, alone or in combination with ruxolitinib, had no effect (Figures 6G-H)
- Both navtemadlin and ruxolitinib decreased Bcl-2 levels with no additional combination affect (data not shown)

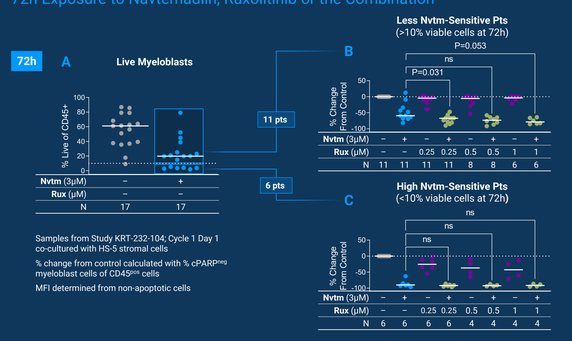
Figure 6. Cell Survival and Protein Expression in MF Patient Samples: 24h or 72h Exposure to Navtemadlin, Ruxolitinib or the Combination



In vivo C_{max}: ruxolitinib 0.25 μM (~5 mg QD); navtemadlin 2.7 μM (~240 mg QD)
% change from control calculated with % cPARP^{neg} myeloblast cells of CD45^{pos} cells; MFI determined from non-apoptotic cells

- MPN-BP myeloblasts showed heterogenous ex vivo sensitivity, with 6/17 samples highly sensitive to navtemadlin alone (<10% viable blasts, Figure 7A) and 11/17 samples sensitive to the combination of navtemadlin added to ruxolitinib (0.25μM) (P=0.03, Figure 7B)

Figure 7. Cell Survival in MPN-BP Patient Samples: 72h Exposure to Navtemadlin, Ruxolitinib or the Combination



Samples from Study KRT-232-104; Cycle 1 Day 1 co-cultured with HS-5 stromal cells
% change from control calculated with % cPARP^{neg} myeloblast cells of CD45^{pos} cells
MFI determined from non-apoptotic cells

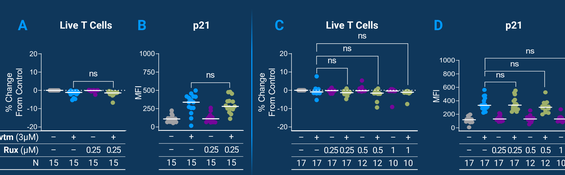
- Navtemadlin combined with ruxolitinib, at various concentrations, had a significant effect on p21 (P=0.03, Figure 8B) and p53 levels (P=0.002, data not shown)
- Mcl-1, Bcl-xL and Bcl-2 levels were not affected by navtemadlin alone or in combination with ruxolitinib (Figures 8C-F)

Figure 8. Protein Expression in MPN-BP Patient Samples: 24h or 72h Exposure to Navtemadlin, Ruxolitinib or the Combination

Samples from study KRT-232-104; Cycle 1 Day 1 co-cultured with stromal cells (HS-5); MFI determined from non-apoptotic cells

- Ruxolitinib added to navtemadlin did not increase apoptosis or suppress p21 expression in T cells (Figures 9A-B) from MF patient or MPN-BP patient samples (Figures 9C-D)

Figure 9. Cell Survival and p21 Expression in T cells from MF or MPN-BP Patient Samples: 72h Exposure to Navtemadlin, Ruxolitinib or the Combination



Samples from patients with myelofibrosis from studies TL-895-201 and KRT-232-113 and patients with MPN-BP from study KRT-232-104. Samples were co-cultured with stromal cells (HS-5). % change from control calculated with % cPARP^{neg} T cells of all T cells; MFI determined from non-apoptotic cells

Conclusion

- Ruxolitinib added to navtemadlin potentiated apoptosis in myeloblasts from MF and MPN-BP patient samples
- This novel combination leveraged complementary biological mechanisms converging on apoptotic cell death by inhibiting p21-mediated cell-cycle arrest and Mcl-1 protein escape without further antagonizing Bcl-xL expression
- Our data showed ruxolitinib synergized with navtemadlin by hastening apoptosis of myeloblasts and inhibited tumor escape, which may improve clinical benefit for ruxolitinib-treated MF patients with suboptimal response to ruxolitinib
- This approach has been explored in the clinic (NCT04485260); emerging clinical data validates the synergistic potential of this combination (EHA 2023, Abstract S210)