Abstract: P991

Title: ADDING NAVTEMADLIN (NVTM) TO RUXOLITINIB (RUX) POTENTIATES APOPTOSIS IN MYELOBLASTS FROM PATIENTS (PTS) WITH MYELOFIBROSIS (MF)

Abstract Type: Poster Presentation

Session Title: Myeloproliferative neoplasms - Biology & Translational Research

Background:

Rux, a Janus kinase (JAK) 1/2 inhibitor can improve splenomegaly and MF-related symptoms; however, the majority of pts experience a suboptimal response with ~50% of pts discontinuing within one year of starting treatment (Harrison 2020). Nvtm, a potent, selective, orally available mouse double minute 2 inhibitor (MDM2i) restores p53 function to modulate Bcl-2 family proteins and drive apoptosis of *TP53* wild-type ($TP53^{WT}$) myeloblasts. Single agent nvtm demonstrated clinically meaningful and disease modifying activity in $TP53^{WT}$ relapsed/refractory MF (Al-Ali 2020; Vachhani 2021).

Efficient MDM2i-driven apoptosis requires overcoming the anti-apoptotic checkpoint p21 (induces cell-cycle arrest) and aberrant JAK2 signaling (upregulates pro-survival Bcl-2 family protein expression [Guo 2014]). We reasoned combining the complimentary mechanisms of nvtm and rux may have synergistic potential to modulate p21-driven cell cycle arrest and dysregulated Bcl-2 signaling mechanisms adopted by MF cells to escape cell death.

Aims:

To assess the combined impact of nvtm and rux in myeloblasts from pts with MF and blast phase myeloproliferative neoplasm (MPN-BP).

Methods:

UKE-1, a $TP53^{WT}$ MF cell line, was used for drug-sensitivity testing and assessing single agent nvtm, rux or synergy of the combination. We also performed drug-sensitivity profiling using multi-parameter flow cytometry on peripheral blood mononuclear cells (PBMC) from pts with MF or MPN-BP (n=15, n=17, respectively). PBMCs were cultured on HS-5 stroma in the presence of clinically relevant concentrations of nvtm (3μM) and rux (0.25-1μM) for 24h or 72h. Antibody panels were used to identify cell subsets; detect apoptosis (cPARP); measure p21, p53, MDM2, and Bcl-2 family proteins in non-apoptotic cell subsets.

Results:

In UKE-1 cells, adding nvtm to rux was synergistic, with near-complete apoptosis observed at 72h. Unexpectedly, the addition of rux diminished the nvtm-mediated induction of p21 (Figure 1). Further, only cells without p21 expression were apoptotic, suggesting rux hindered the protective role of p21 in p53-mediated apoptosis.

Extending this finding to primary MF cells, adding nvtm to rux increased apoptosis in myeloblasts compared with nvtm alone (p=0.04 at 72h, Figure 2A, 2B). Prior to apoptosis, nvtm-mediated p21 induction was again observed, but was significantly inhibited by the combination (p=0.002, Figure 2C, 2D). Interestingly, p21 suppression by rux added to nvtm was not observed in gated B or T cells from pts. Importantly, the combination treatment also reduced pro-survival Bcl-2 family proteins, including near-complete inhibition of Mcl-1 expression compared to nvtm or rux alone (p=0.001, Figure 2E-H). In contrast, MPN-BP myeloblasts were heterogenous with 6 of 17 samples highly sensitive to nvtm alone (<10% viable blasts at 72h), and the remaining 11 sensitive to combined nvtm and rux (p=0.03). Levels of p21 and Mcl-1 decreased at higher rux exposure [0.25-1μM] in MPN-BP cells compared to primary MF cells.

Summary/Conclusion:

Rux potentiates nvtm-driven apoptosis in myeloblasts from MF and MPN-BP pts. The combination leverages complementary mechanisms converging on apoptotic cell death by inhibiting transient p21-mediated cell-cycle arrest, Mcl-1 and Bcl-xL protein expression. Our data show how rux can synergize with nvtm to hasten apoptosis and reduce tumor escape, which may offer improved clinical benefit for rux treated MF pts with suboptimal response.

Figure 1: Intracellular Flow Cytometry of UKE-1 Cells After 24h Drug Exposure

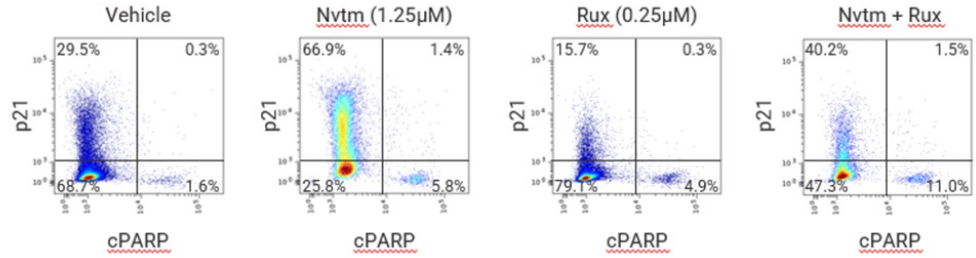
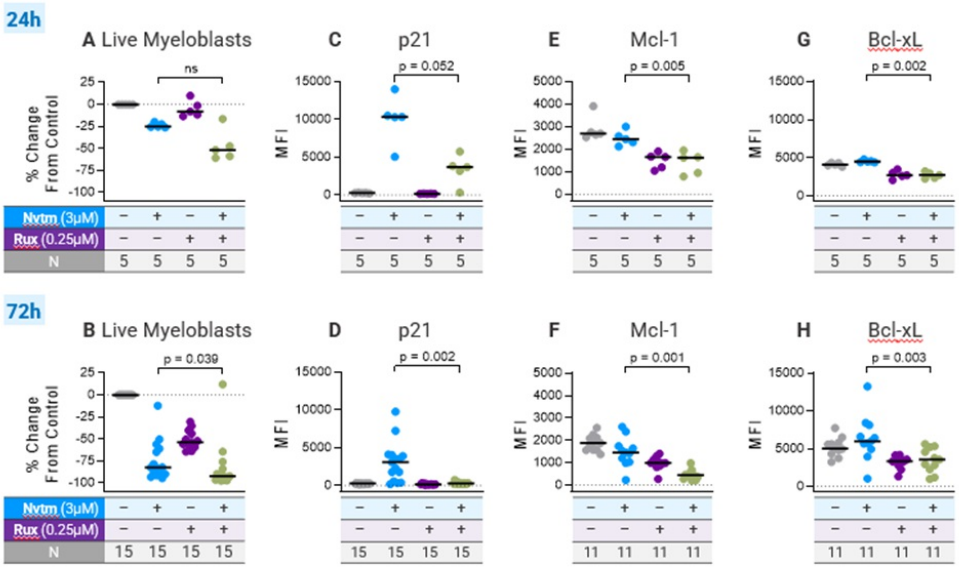


Figure 2: Apoptosis and Protein Expression in MF Pt Samples After 24h and 72h Drug Exposure



Abbreviations: MF, myelofibrosis; MFI, median fluorescent intensity; Pt, patient.

Keywords: Ruxolitinib, p21, p53, Myeloproliferative disorder