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631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Activity of Selinexor As a Single Agent and Synergistic Activity with Approved/Investigational Myelofibrosis Therapies in Vitro

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) that commonly harbors acquired somatic gene mutations in *JAK2*, *CALR* or *MPL* leading to constitutive JAK/STAT activation . Despite the current standard of care, the JAK1/2 inhibitor (JAKi) ruxolitinib (RUX), a significant unmet need remains for patients (pts) with MF, due to lack of response or JAKi resistance. Selinexor (SEL) is an investigational, oral XPO1 inhibitor that may inhibit multiple pathways relevant in MF including JAK/STAT, ERK, and AKT. Previously, we reported that 60 mg SEL in combination with RUX in pts with JAKi-naïve MF resulted in rapid, deep, and sustained spleen responses even in pts receiving suboptimal RUX doses, suggesting synergy between SEL and RUX while maintaining a generally manageable safety profile (Ali et *al.*, ASCO 2023; Ali et *al.*, AACR 2023). Here, we investigated the combinatorial effects of SEL with 5 approved/investigational MF therapeutic agents on human cell lines, then evaluated relevant downstream pathways impacted by SEL +/- RUX.

Methods: *JAK2* ^{V617F} mutant cell lines HEL (*TP53* ^{M113K}), UKE-1 (*TP53* ^{WT}), MUTZ-8 (*TP53* ^{WT}), and *JAK2* ^{WT} ELF-153 (*TP53* ^{I251N}) cells were treated with SEL alone or in combination with RUX, pacritinib (PAC), momelotinib (MMB), pelabresib (PELA), or navitoclax (NAV). Compounds were added to $1x10^4$ cells in a 1:3 or 1:2 serial dilution, then cell viability was assessed 72 hours post-exposure. Single-agent non-linear regressions were used to determine inhibitory concentrations (IC), and drug synergy was evaluated using Synergy Finder 3.0 (Bliss). HEL (*JAK2* ^{V617F}) and ELF-153 (*JAK2* ^{wt}) cells were used for mechanistic studies including western blots and RNA sequencing.

Results: Single agent SEL reduced cell viability in all cell lines tested, independent of *JAK2* mutation status: HEL (IC_{50} 320nM), UKE-1 (320 nM), MUTZ-8 (12nM) and ELF-153 (570 nM) cells. SEL antiproliferative activity was more potent than RUX (IC_{50} range 1-64.6 μM), MMB (1.9-27.8 μM), NAV (0.165-51.4 μM) and PELA (7.5-65.9 μM). When determined by Bliss scores, evidence of synergy was observed in the following co-treatments: SEL + RUX in HEL (10.8) and UKE-1 (11.9) cells; SEL + PELA in HEL (9.9) and UKE-1 (7.7); SEL + MMB (16.1) and NAV (12.4) in HEL (**Figure 1**). Additive effects were observed: SEL + RUX in MUTZ-8 and ELF-153 (*JAK2* ^{wt}) cells; SEL + MMB in UKE-1, MUTZ-8 ELF-153 cells; SEL + PAC in HEL and ELF-153 cells; SEL+ PELA in UKE-1, MUTZ-8 and ELF-153 cells; SEL+NAV in UKE-1 and ELF-153 cells.

Mechanistically, SEL and the SEL + RUX combo both induced XPO1 protein degradation to a similar extent. The SEL + RUX combination in HEL cells resulted in a pro-apoptotic effect via decreased MCL-1 protein ($P \leq 0.0001$) and increased cleaved PARP vs RUX treatment. Preliminary analysis of mRNA transcript alterations after SEL + RUX treatment showed a modulation of expression of genes related to proliferation (*MYC*), senescence (*CDKN1A/B)* and cell cycle (*CYCLIND1/A2/B1*).

A RUX-resistant HEL cell clone was generated via culture with increasing RUX, resulting in a >10-fold increase in RUX IC_{50}. The SEL IC_{50} of the resistant clone was not changed compared to parental cells. RUX-resistant HEL cells showed downregulation of several pathways including MYC, G2M checkpoint, ribosomal biogenesis, proteasome, spliceosome, and nuclear export when compared to parental HEL cells. SEL had synergistic or additive effects with MF agents in this resistant subclone.

Conclusion: In a panel of MPN-derived cells with or without *JAK2* ^{V617F}, SEL showed single-agent antiproliferative activity, and synergism with other MF therapeutics at clinically achievable concentrations through regulation of XPO1, JAK/STAT signaling, and apoptosis/senescence regulators. In addition to positive Phase 1 clinical data in pts with JAKi-naïve MF who received SEL with RUX, these preclinical data support synergistic activity between SEL and other MF therapies, suggesting the potential for SEL as a backbone for novel treatment combinations for MF.

Disclosures Maloof: *Karyopharm:* Current Employment. **Wu:** *Karyopharm:* Current Employment. **Ellero:** *Karyopharm:* Current Employment. **Walker:** *Karyopharm:* Current Employment. **Kye:** *Karyopharm:* Current Employment. **Taverna:** *Karyopharm:* Current Employment.

Line	Selinexor Range	Pacritinib Range	Ruxolitinib Range	Momelotinib Range	Pelabresib Range	Navitoclax Range
HEL $JAK2^{MUT/MUT}$	0-0.2μM	0-0.8μM	0-1.5μM	0-3.5μM	0-16μM	0-0.5μM
	Bliss	0.46	10.82	16.14	9.91	12.44
UKE-1 $JAK2^{MUT/MUT}$	0-0.1μM	0-3μM	0-20μM	0-2.5μM	0-16μM	0-30μM
	Bliss	-8.4	11.89	-0.31	7.65	4.84
MUTZ-8 $JAK2^{MUT/MUT}$	0-6μM	0-0.5μM	0-6μM	0-8μM	0-8μM	0-0.2μM
	Bliss	-6.3	-6.93	-6.67	-4.56	-0.2
ELF-153 $JAK2^{WT/WT}$	0-4μM	0-0.8μM	0-20μM	0-20μM	0-20μM	0-2.5μM
	Bliss	5.0	3.01	3.05	6.6	6.33

Figure 1. Synergy and additivity of selinexor in combinations with approved/investigational myelofibrosis drugs across human transformed cell lines ± $JAK2^{V617F}$ mutation.

Bliss scores defined as the following: Synergy ≥10; Additivity 0-10; Antagonism ≤10

Figure 1

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