Abstract: #123

Activity of Selinexor as a Single Agent and Synergistic Activity With Approved/Investigational Myelofibrosis Therapies in Vitro

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Introduction

- Myelofibrosis (MF) is a heterogenous, progressive, and fatal disease with the underlying biological hallmarks of aberrant blood and bone marrow differentiation, increased cytokine production and inflammation, bone marrow fibrosis with presence of driver mutations (e.g. *JAK2*, *CALR*, and *MPL*), and dysregulated cell proliferation of megakaryocytes/granulocytes.[1,2]
- Current therapies, including ruxolitinib and other Janus kinase inhibitors (JAKis), primarily target the JAK/STAT pathway commonly overactivated in MF, but their ability to modify the disease and impact overall survival is unclear.[3]
  - <50% of patients achieve spleen volume reduction of 35% from baseline (SVR35) and total symptom score reduction of 50% from baseline (TSS50) with ruxolitinib at Week 24,[3] and the probability of maintaining response declines as early as Week 12 following response in patients who achieved SVR35 with ruxolitinib.[4]
- Selinexor is an investigational oral exportin 1 (XPO1) inhibitor with pro-apoptotic and anti-inflammatory properties that may impact both JAK and non-JAK pathways.[5,6]
- Previously, we reported that rapid, deep, and sustained spleen volume reduction and robust symptom improvement were observed with selinexor plus ruxolitinib independent of ruxolitinib dose, even in patients receiving suboptimal ruxolitinib doses, suggesting synergy between selinexor and ruxolitinib.[7]
  - See the accompanying Poster 130: Tantravahi, et al. Selinexor Plus Ruxolitinib in JAK Inhibitor Treatment-Naïve Patients With Myelofibrosis: Long-Term Follow-up and Disease Modification From XPORT-MF-034.
- Here, we investigated the in vitro combinatorial effects of selinexor with five approved or investigational MF therapeutic agents in human cell lines. We also evaluated the activity of selinexor with and without ruxolitinib on downstream pathways that may be relevant to the disease mechanism of MF.

Methods

- A panel of human transformed cell lines originating from patients with myeloproliferative neoplasms (MPNs) with and without *JAK2*^{V617F} and *TP53* mutations was used to determine synergy between selinexor and other approved or investigational MF therapies. The characteristics of cell lines are shown in Table 1.

Table 1. Characteristics of Cell Lines

Name	Origin	Mutation status	
		JAK2	*TP53*
HEL	Erythroleukemia in relapse (myeloblast)	V617F (hom)	M133L (hom)
UKE1	Essential thrombocythemia to acute myeloid leukemia	V617F (hom)	wt
MUTZ-8	Myelodysplastic syndrome to acute myeloid leukemia	V617F (hom)	wt
ELF-153	Acute megakaryocytic leukemia post myelofibrosis	wt	I251N (hom)

- To assess efficacy and synergy, cells were exposed to either dimethyl sulfoxide (DMSO), selinexor alone, or selinexor in combination with one of the following treatments: Ruxolitinib, momelotinib, pacritinib (all JAKis), pelabresib (investigational bromodomain and extra-terminal domain inhibitor), or navitoclax (investigational BLC2 inhibitor). The combinatorial effects of selinexor with other MF agents were evaluated with the CellTiter-Glo® assay (Promega, Madison, WI, USA) following a 72-hour exposure. Synergy or additivity was determined using Synergy Finder 3.0 based on the Bliss model.[8]
- To evaluate the mechanistic relevance of selinexor and ruxolitinib (alone and in combination) HEL, UKE-1, and ELF-153 cells were treated for 24 hours at concentrations lower than the IC_{50}. Expression of proteins related to, or involved in, apoptosis, cell cycle regulation, JAK/STAT pathway and XPO1 cargoes was assessed by western blotting. Cell cycle analysis was performed using flow cytometry.
- To investigate if selinexor in combination with other MF agents remains effective in an in vitro model of ruxolitinib resistance, a ruxolitinib-resistant HEL cell clone was generated by continuous exposure of HEL cells to increasing amounts of ruxolitinib beginning at IC_{10} and titrating upwards. Ruxolitinib resistance was defined as IC_{50} ≥10× higher than the parental cell line.
- Transcriptional profiling of ruxolitinib-resistant cells was carried out in triplicate using RNA sequencing (Discovery Life Sciences, Huntsville, AL). Gene set enrichment analysis (GSEA) compared resistant and parental cells using Wald statistics for all expressed genes and MSigDB Hallmark, KEGG, and GO canonical pathway sets.

Results

Single-Agent Cell Viability and Synergy

Table 2. IC_{50} Cell Viability of Single Agents

Line	Selinexor IC_{50}	Ruxolitinib IC_{50}	Momelotinib IC_{50}	Pacritinib IC_{50}	Navitoclax IC_{50}	Pelabresib IC_{50}
HEL *JAK2*$^{MUT/MUT}$	0.32 µM	1 µM	2.7 µM	0.82 µM	0.43 µM	2.2 µM
UKE-1 *JAK2*$^{MUT/MUT}$	0.32 µM	2.3 µM	1.3 µM	0.32 µM	0.054 µM	1.7 µM
MUTZ-8 *JAK2*$^{MUT/MUT}$	0.012 µM	3 µM	3.8 µM	0.52 µM	0.028 µM	4.5 µM
ELF-153 *JAK2*$^{WT/WT}$	1.8 µM	64.6 µM	6.2 µM	0.82 µM	3.99 µM	1.6 µM

- Selinexor as a single agent reduced cell viability in all cell lines tested, independent of *JAK2* and *TP53* mutation status, and was more potent than ruxolitinib, momelotinib, navitoclax, and pelabresib (Table 2).

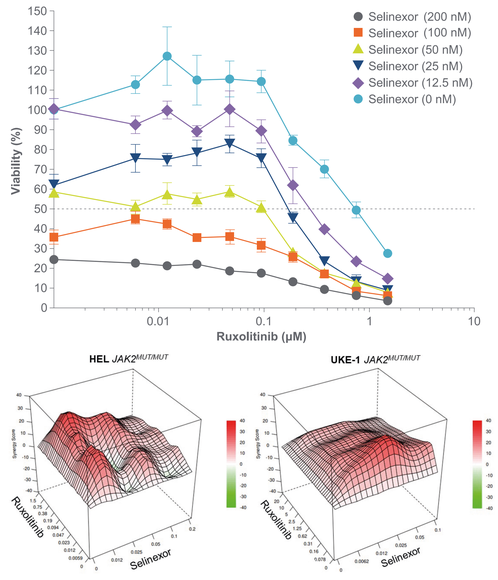
Table 3. Synergy and Additivity of Selinexor in Combination With Clinically Achievable Doses of Approved or Investigational Myelofibrosis Agents

Line	Selinexor range	Ruxolitinib range	Momelotinib range	Pacritinib range	Navitoclax range	Pelabresib range
HEL *JAK2*$^{MUT/MUT}$	0–0.2 µM	0–1.5 µM	0–3.5 µM	0–0.8 µM	0–0.5 µM	0–16 µM
	Bliss	10.82	16.14	10.4	12.44	9.91
UKE-1 *JAK2*$^{MUT/MUT}$	0–0.1 µM	0–20 µM	0–2.5 µM	0–0.3 µM	0–30 µM	0–16 µM
	Bliss	11.89	–0.31	17.5	4.84	7.65
MUTZ-8 *JAK2*$^{MUT/MUT}$	0–6 µM	0–6 µM	0–8 µM	0–0.5 µM	0–0.5 µM	0–8 µM
	Bliss	–6.93	–6.67	–0.23	–0.2	–4.56
ELF-153 *JAK2*$^{WT/WT}$	0–4 µM	0–20 µM	0–20 µM	0–0.8 µM	0–2.5 µM	0–20 µM
	Bliss	3.01	3.05	15.8	6.33	6.6

Bliss scores were defined as follows: Synergy ≥10 (blue), additivity between –10 and 10 (black).

- When selinexor was given in combination with other agents, evidence of both synergy and additivity was observed, except for in combination with pelabresib where only additivity was observed (Table 3).

Figure 1. Effect of Increasing Selinexor Concentrations on Ruxolitinib IC_{50} in HEL Cells (A) and Three Dimensional Bliss Plot (B)

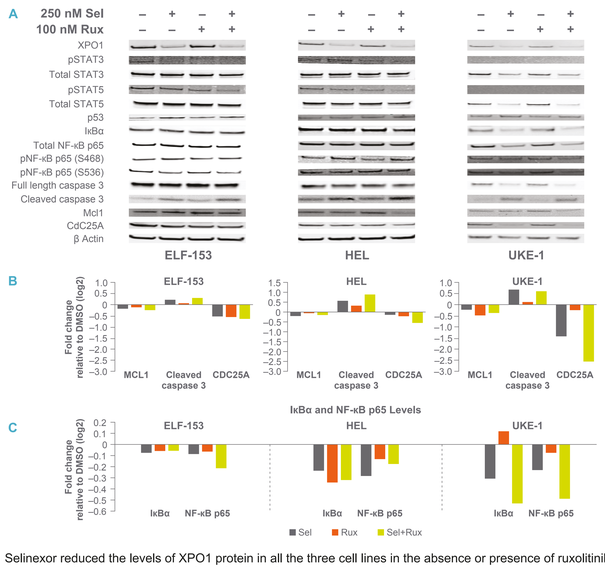


Red indicates areas of synergy. Color intensity corresponds to degree of synergy. White indicates areas of additivity. Green indicates areas of antagonism.

- In HEL cells, synergic combinatory effect was seen with dose-dependent reduction in ruxolitinib IC_{50} with increasing selinexor concentrations (Figure 1A and 1B together with UKE-1 cells that show a peak in synergy scores at low doses of ruxolitinib).

Selinexor Impact on JAK/STAT, Cell Cycle, and NF-κB Signaling Pathways

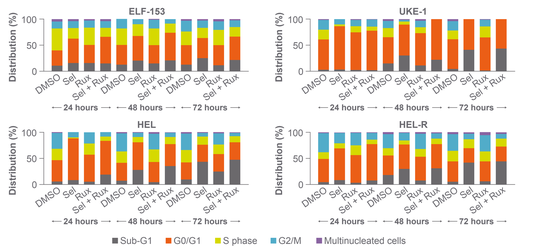
Figure 2. Western Blotting (A) and Densitometry (B and C) in ELF-153, HEL, and UKE-1 Cells Treated With Selinexor, Ruxolitinib, or Combination for 24 Hours



- Selinexor reduced the levels of XPO1 protein in all the three cell lines in the absence or presence of ruxolitinib, consistent with the known mechanism of action of selinexor (Figure 2A).
- Selinexor plus ruxolitinib combination treatment negatively impacted the phosphorylation status of STAT3 and STAT5 irrespective of *JAK2* mutation status in two of the cell lines (ELF-153, HEL) tested. In the *TP53* wild-type cell line (UKE1), selinexor reduced the expression of total STAT3 and STAT5 (Figure 2A).
- Attenuation of MCL-1, CDC25A expression and increased levels of cleaved caspase 3 were observed in the selinexor plus ruxolitinib-treated groups for all the three cell lines (Figure 2A and B).
- Levels of IκBα and total NF-κB p65 were reduced in selinexor single and combination samples, and the reduction was more pronounced in the *TP53* wild-type cell line (UKE1) (Figure 2A and C).

Selinexor Impact on Cell Cycle and Apoptosis

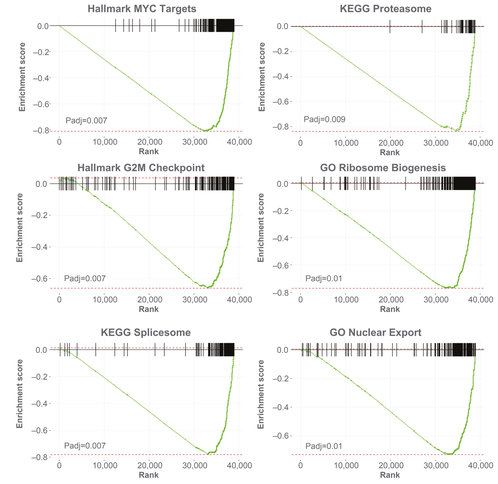
Figure 3. Cell Cycle Analysis in ELF-153, UKE-1, HEL, and HEL-R Cells Treated With Selinexor, Ruxolitinib, or Combination for 24 Hours



- An increase in the relative number of cells in G0/G1 together with a relative decrease in the number of cells in S phase were seen in the selinexor and combination treatment groups after 24 hours of exposure, with the most prominent G0/G1 arrest occurring in p53 wild-type cells, UKE-1 (Figure 3).
- Increase in sub-G1 phase, indicating apoptosis, was seen in a time-dependent manner with both selinexor and combination treatment (Figure 3).
- Selinexor treatment showed a similar cell cycle profile to combination treatment in all cell lines, which may indicate that selinexor is driving the effects in combination therapy (Figure 3).
- Interestingly, CDC25A, which positively regulates G0/G1 cell cycle progression, was reduced by selinexor and combination treatment, with a more pronounced reduction in UKE-1 cells (Figure 2A and B).

JAKi Sensitive and Resistant Cells Transcriptomics

Figure 4. Enrichment Scores for Cells With Induced Resistance to Ruxolitinib Versus Parental HEL Cells



The vertical black lines at the top of each enrichment score plot show a marked rank order list of differentially expressed genes for those genes that are included in the queried pathway. The green curve shows the running sum statistic as the analysis walks down the rank order list, with the score at the peak representing the enrichment score for the gene set.

- The potential mechanisms facilitating ruxolitinib resistance in HEL cell lines were investigated using bulk RNA sequencing.
  - The resistance was mediated by multiple indirect signaling pathway changes at the RNA level rather than by direct *JAK2* mutations or only JAK/STAT signaling changes.
    - GSEA showed that compared with parental HEL cells, several pathways were downregulated in ruxolitinib-resistant HEL cells, including MYC, G2M checkpoint, ribosomal biogenesis, proteasome, spliceosome, and nuclear export (Figure 4).

Table 4. Synergy and Additivity of Selinexor in Combination With Clinically Achievable Doses of Approved or Investigational Myelofibrosis Agents in Ruxolitinib Sensitive (HEL) and Resistant (HEL-R) cells

Line	Selinexor range	Ruxolitinib range	Momelotinib range	Navitoclax range	Pelabresib range
HEL *JAK2*$^{MUT/MUT}$	0–0.2 µM	0–1.5 µM	0–3.5 µM	0–0.5 µM	0–16 µM
	Bliss	10.82	16.14	12.44	9.91
HEL-R *JAK2*$^{MUT/MUT}$	0–0.2 µM	0–16 µM	0–2 µM	0–1 µM	0–4 µM
	Bliss	13.27	2.00	–5.18	0.69

Bliss scores were defined as follows: Synergy ≥10 (blue), additivity between –10 and 10 (black).

- Selinexor maintained its antiproliferative effect in ruxolitinib-resistant cells (IC_{50} of 400 nm vs 320 nm in parental cell line), its impact on cell cycle (Figure 3), and its effectiveness when used in combination with other agents (Table 4).

Conclusions

- In a panel of MPN-derived cells with or without *JAK2*^{V617F} and *TP53* mutations, selinexor was shown to have single-agent antiproliferative activity and synergism with other therapeutic agents for MF at clinically achievable concentration, indicating its potential as a backbone for novel treatment combinations for MF.
- The antiproliferative effect of selinexor and its synergy and additivity with other MF agents was independent of ruxolitinib resistance.
- Selinexor induced G0/G1 cell cycle arrest and cell apoptosis through upregulation of caspase 3 cleavage and through inhibition of the NF-kB pathway, as seen by reduction of downstream proteins IκBα and NF-κB p65.
  - Selinexor combination with ruxolitinib enhanced these effects.
- These nonclinical data provide further evidence that XPO1 inhibition by selinexor is potentially synergistic with ruxolitinib, supporting clinical results from the Phase 1 trial that showed rapid and deep spleen response and robust symptom improvement associated with selinexor in combination with ruxolitinib in JAKi-naïve patients with MF.[7,9]
- Selinexor has the potential to be combined with multiple other agents, targeting MF through both JAK and non-JAK pathways.
- A Phase 3 trial of selinexor in combination with ruxolitinib (NCT04562389) and a Phase 2 of selinexor monotherapy (NCT05980806) are currently evaluating selinexor as the backbone of therapy in JAKi-naïve patients with MF.

Abbreviations

BLC2, B-cell lymphoma 2; CALR, calreticulin; CDC25A, cell division cycle 25 homolog A; DMSO, dimethyl sulfoxide; GO, gene ontology; GSEA, gene set enrichment analysis; HEL-R, ruxolitinib-resistant HEL; hom, homozygous; IC_{50}, XX% inhibitory concentration; IκBα, inhibitor of nuclear factor kappa-B kinase subunit alpha; JAK, Janus kinase; JAKi, Janus kinase inhibitor; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCL1, myeloid cell leukemia 1; MF, myelofibrosis; MPL, myeloproliferative leukemia virus; MPN, myeloproliferative neoplasm; MSigDB, Molecular Signatures Database; MUT, mutant; MYC, master regulator of cell cycle entry and proliferative metabolism; NF-κB, nuclear factor k-light-chain-enhancer of activated B cells; p53/65, tumor suppressor protein 53/65; padj, adjusted p-value; pNF-κB, phosphorylated nuclear factor k-light-chain-enhancer of activated B cells; pSTAT, phosphorylated signal transducer and activator of transcription; RNA, ribonucleic acid; Rux, ruxolitinib; Sel, selinexor; STAT, signal transducer and activator of transcription; SVR35, spleen volume reduction of 35% from baseline; TP53, tumor suppressor protein 53 gene; TSS50, reduction in total symptom score of 50% from baseline; wt, wild type; XPO1, exportin 1.

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Disclosures

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